REPORT #
RRTAC 88-3

Revegetation Of Oil Sands Tailings: Growth Improvement Of Silver-Berry And Buffalo-Berry By Inoculation With Mycorrhizal Fungi And N_2-Fixing Bacteria

Heritage Fund

Alberta
LAND CONSERVATION AND
RECLAMATION COUNCIL
Reclamation Research
Technical Advisory Committee

Report No. RRTAC 88-3

REVEGETATION OF OIL SANDS TAILINGS: GROWTH IMPROVEMENT OF SILVER-BERRY AND BUFFALO-BERRY BY INOCULATION WITH MYCORRHIZAL FUNGI AND N_2-FIXING BACTERIA

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Prepared for

The Oil Sands Reclamation Research Program

of

THE LAND CONSERVATION AND RECLAMATION COUNCIL

(Reclamation Research Technical Advisory Committee)

1988

STATEMENT OF OBJECTIVE

The recommendations and conclusions in this report are those of the authors and not those of the Alberta Government or its representatives.

This report is intended to provide government and industry staff with up-to-date technical information to assist in the development of guidelines and operating procedures. The report is also available to the public so that interested individuals similarly have access to the best available information on land reclamation topics.

ALBERTA'S RECLAMATION RESEARCH PROGRAM

The regulation of surface disturbances in Alberta is the responsibility of the Land Conservation and Reclamation Council. The Council executive consists of a Chairman from the Department of Forestry, Lands and Wildlife. Among other functions, the Council oversees programs for reclamation of abandoned disturbances and reclamation research. The Reclamation Research Program was established to provide answers to the many practical questions which arise in reclamation. Funds for implementing both the operational and research programs are drawn from Alberta's Heritage Savings Trust Fund.

To assist in technical matters related to the development and administration of the Research Program, the Council appointed the Reclamation Research Advisory Committee (RRTAC). The Committee first met in March 1978 and consists of eight members representing the Alberta Departments of Agriculture, Energy, Forestry, Lands and Wildlife, Environment and the Alberta Research Council. The Committee meets regularly to update research priorities, review solicited and unsolicited research proposals, arrange workshops and otherwise act as a referral and coordinating body for Reclamation Research.

Additional information on the Reclamation Research Program may be obtained by contacting:

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This report may be cited as: Visser, S. and R.M. Danielson, 1988. Revegetation of Oil Sands Tailings: Growth Improvement of Silver-Berry and Buffalo-Berry by Inoculation with Mycorrhizal Fungi and N_2-Fixing Bacteria. Alberta Land Conservation and Reclamation Council Report #RRTAC 88-3. 98 pp.

Additional copies may be obtained from:

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RECLAMATION RESEARCH REPORTS

** 1. RRTAC 80-3: The Role of Organic Compounds in Salinization of Plains Coal Mining Sites. N.S.C. Cameron et al. 46 pp.

DESCRIPTION: This is a literature review of the chemistry of sodic mine spoil and the changes expected to occur in groundwater.

** 2. RRTAC 80-4: Proceedings: Workshop on Reconstruction of Forest Soils in Reclamation. P.F. Ziemkiewicz, S.K. Takyi, and H.F. Regier. 160 pp.

DESCRIPTION: Experts in the field of forestry and forest soils report on research relevant to forest soil reconstruction and discuss the most effective means of restoring forestry capability of mined lands.

N/A 3. RRTAC 80-5: Manual of Plant Species Suitability for Reclamation in Alberta. L.E. Watson, R.W. Parker, and P.F. Polster. 2 vols, 541 pp.

DESCRIPTION: Forty-three grass, fourteen forb, and thirty-four shrub and tree species are assessed in terms of their fitness for use in Reclamation. Range maps, growth habit, propagation, tolerance, and availability information are provided.

N/A 4. RRTAC 81-2: 1980 Survey of Reclamation Activities in Alberta. D.G. Walker and R.L. Rothwell. 76 pp.

DESCRIPTION: This survey is an update of a report prepared in 1976 on reclamation activities in Alberta, and includes research and operational reclamation, locations, personnel, etc.

N/A 5. RRTAC 81-3: Proceedings: Workshop on Coal Ash and Reclamation. P.F. Ziemkiewicz, R. Stien, R. Leitch, and G. Lutwick. 253 pp.

DESCRIPTION: Presents nine technical papers on the chemical, physical and engineering properties of Alberta fly and bottom ashes, revegetation of ash disposal sites and use of ash as a soil amendment. Workshop discussions and summaries are also included.

N/A 6. RRTAC 82-1: Land Surface Reclamation: An International Bibliography. H.P. Sims and C.B. Powter. 2 vols, 292 pp.

DESCRIPTION: Literature to 1980 pertinent to reclamation in Alberta is listed in Vol. 1 and is also on the University of Alberta computing system. Vol. 2 comprises the keyword index and computer access manual.

N/A 7. RRTAC 82-2: A Bibliography of Baseline Studies in Alberta: Soils, Geology, Hydrology and Groundwater. C.B. Powter and H.P. Sims. 97 pp.

DESCRIPTION: This bibliography provides baseline information for persons involved in reclamation research or in the preparation of environmental impact assessments. Materials, up to date as of December 1981, are available from the Alberta Environment Library.

N/A 8. RRTAC 83-1: Soil Reconstruction Design for Reclamation of Oil Sand Tailings. Monenco Consultants Ltd. 185 pp.

DESCRIPTION: Volumes of peat and clay required to amend oil sand tailings were estimated based on existing literature. Separate soil prescriptions were made for spruce, jack pine, and herbaceous cover types. The estimates form the basis of field trials.

N/A 9. RRTAC 83-3: Evaluation of Pipeline Reclamation Practices on Agricultural Lands in Alberta. Hardy Associates (1978) Ltd. 205 pp.

DESCRIPTION: Available information on pipeline reclamation practices was reviewed. A field survey was then conducted to determine the effects of pipe size, age, soil type, construction method, etc. on resulting crop production.

N/A 10. RRTAC 83-4: Proceedings: Effects of Coal Mining on Eastern Slopes Hydrology. P.F. Ziemkiewicz. 123 pp.

DESCRIPTION: Technical papers are presented dealing with the impacts of mining on mountain watersheds, their flow characteristics and resulting water quality. Mitigative measures and priorities were also discussed.

N/A 11. RRTAC 83-5: Woody Plant Establishment and Management for Oil Sands Mine Reclamation. Techman Engineering Ltd. 124 pp.

DESCRIPTION: This is a review and analysis of information on planting stock quality, rearing site preparation, planting and procedures necessary to ensure survival of trees and shrubs in oil sand reclamation.

*** 12. RRTAC 84-1: Land Surface Reclamation: A Review of International Literature. H.P. Sims, C.B. Powter, and J.A. Campbell. 2 vols, 1549 pp.

DESCRIPTION: Nearly all topics of interest to reclamation including mining methods, soil amendments, revegetation, propagation and toxic materials are reviewed in light of the international literature.

** 13. RRTAC 84-2: Propagation Study: Use of Trees and Shrubs for Oil Sand Reclamation. Techman Engineering Ltd. 58 pp.

DESCRIPTION: This report evaluates and summarizes all available published and unpublished information on large-scale propagation methods for shrubs and trees to be used in oil sand reclamation.

* 14. RRTAC 84-3: Reclamation Research Annual Report - 1983. P.F. Ziemkiewicz. 42 pp.

DESCRIPTION: This report details the Reclamation Research Program indicating priorities, descriptions of each research project, researchers, results and expenditures.

** 15. RRTAC 84-4: Soil Microbiology in Land Reclamation. D. Parkinson, R.M. Danielson, C. Griffiths, S. Visser, and J.C. Zak. 2 vols, 676 pp.

DESCRIPTION: This is a collection of five reports dealing with re-establishment of fungal decomposers and mycorrhizal symboints in various amended spoil types.

** 16. RRTAC 85-1: Proceedings: Revegetation Methods for Alberta's Mountains and Foothills. P.F. Ziemkiewicz. 416 pp.

DESCRIPTION: Results of long-term experiments and field experience on species selection, fertilization, reforestation, topsoiling, shrub propagation and establishment are presented.

* 17. RRTAC 85-2: Reclamation Research Annual Report - 1984. P.F. Ziemkiewicz. 29 pp.

DESCRIPTION: This report details the Reclamation Research Program indicating priorities, descriptions of each research project, researchers, results and expenditures.

** 18. RRTAC 86-1: A Critical Analysis of Settling Pond Design and Alternative Technologies. A. Somani. 372 pp.

DESCRIPTION: The report examines the critical issue of settling pond design and sizing and alternative technologies.

** 19. RRTAC 86-2: Characterization and Variability of Soil Reconstructed after Surface Mining in Central Alberta. T.M. Macyk. 146 pp.

DESCRIPTION: Reconstructed soils representing different materials handling and replacement techniques were characterized and variability in chemical and physical properties was assessed. The data obtained indicate that reconstructed soil properties are determined largely by parent material characteristics and further tempered by materials handling procedures. Mining tends to create a relatively homogeneous soil landscape in contrast to the mixture of diverse soils found before mining.

* 20. RRTAC 86-3: Generalized Procedures for Assessing Post-Mining Groundwater Supply Potential in the Plains of Alberta - Plains Hydrology and Reclamation Project. M.R. Trudell and S.R. Moran. 30 pp.

DESCRIPTION: In the Plains region of Alberta, the surface mining of coal generally occurs in rural, agricultural areas in which domestic water supply requirements are met almost entirely by groundwater. Consequently, an important aspect of the capability of reclaimed lands to satisfy the needs of a residential component is the post-mining availability of groundwater. This report proposes a sequence of steps or procedures to identify and characterize potential post-mining aquifers.

** 21. RRTAC 86-4: Geology of the Battle River Site: Plains Hydrology and Reclamation Project. A Maslowski-Schutze, R. Li, M. Fenton and S.R. Moran. 86 pp.

DESCRIPTION: This report summarzies the geological setting of the Battle River study site. It is designed to provide a general understanding of geological conditions adequate to establish a framework for hydrogeological and general reclamation studies. The report is not intended to be a detailed synthesis such as would be required for mine planning purposes.

** 22. RRTAC 86-5: Chemical and Mineralogical Properties of Overburden: Plains Hydrology and Reclamation Program. A. Maslowski-Schutze. 71 pp.

DESCRIPTION: This report describes the physical and mineralogical properties of overburden materials in an effort to identify individual beds within the bedrock overburden that might be significantly different in terms of reclamation potential.

* 23. RRTAC 86-6: Post-Mining Groundwater Supply at the Battle River Site: Plains Hydrology and Reclamation Project. M.R. Trudell, G.J. Sterenberg and S.R.-Moran. 49 pp.

DESCRIPTION: The report deals with the availability of water supply in or beneath cast overburden at the Battle River Mining area in east-central Alberta to support post-mining land use. Both groundwater quantity and quality are evaluated.

* 24. RRTAC 86-7: Post-Mining Groundwater Supply at the Highvale Site: Plains Hydrology and Reclamation Project. M.R. Trudell. 25 pp.

DESCRIPTION: This report evaluates the availability of water supply in or beneath cast overburden to support post-mining land use, including both quantity and quality considerations. The study area is the Highvale mining area in west-central Alberta.

* 25. RRTAC 86-8: Reclamation Research Annual Report - 1985. P.F. Ziemkiewicz. 54 pp.

DESCRIPTION: This report details the Reclamation Research Program indicating priorities, descriptions of each research project, researchers, results and expenditures.

** 26. RRTAC 86-9: Wildlife Habitat Requirements and Reclamation Techniques for the Mountains and Foothills of Alberta. J.E. Green, R.E. Salter and D.G. Walker. 285 pp.

DESCRIPTION: This report presents a review of relevant North American literature on wildlife habitats in mountain and foothills biomes, reclamation techniques, potential problems in wildlife habitat reclamation, and potential habitat assessment methodologies. Four biomes (Alpine, Subalpine, Montane, and Boreal Uplands) and 10 key wildlife species (snowshoe hare, beaver, muskrat, elk, moose, caribou, mountain goat, bighorn sheep, spruce grouse, and white-tailed ptarmigan) are discussed.

** 27. RRTAC 87-1: Disposal of Drilling Wastes. L.A. Leskiw, E. Reinl-Dwyer, T.L. Dabrowski, B.J. Rutherford and H. Hamilton. 210 pp.

DESCRIPTION: Current drilling waste disposal practices are reviewed and criteria in Alberta guidelines are assessed. The report also identifies research needs and indicates mitigation measures. A manual included provides a decision-making flowchart to assist in selecting methods of environmentally safe waste disposal.

** 28. RRTAC 87-2: Minesoil and Landscape Reclamation of the Coal Mines in Alberta's Mountains and Foothills. A.W. Fedkenheuer, L.J. Knapik, and D.G. Walker. 174 pp.

DESCRIPTION: This report reviews current reclamation practices with regard to site and soil reconstruction and re-establishment of biological productivity. It also identifies research needs in the Mountain-Foothills area.

** 29. RRTAC 87-3: Gel and Saline Drilling Wastes in Alberta: Workshop Proceedings. D.A. Lloyd (compiler). 218 pp.

DESCRIPTION: Technical papers were presented which describe: the mud systems used and their purpose; industrial constraints; government regulations, procedures and concerns; environmental considerations in waste disposal; and toxic constituents of drilling wastes. Answers to a questionnaire distributed to participants are included in an appendix.

* 30. RRTAC 87-4: Reclamation Research Annual Report - 1986. 50 pp.

DESCRIPTION: This report details the Reclamation Research Program indicating priorities, descriptions of each research project, researchers, results and expenditures.

* 31. RRTAC 87-5: Review of the Scientific Basis of Water Quality Criteria for the East Slope Foothills of Alberta. Beak Associates Consulting Ltd. 46 pp.

DESCRIPTION: The report reviews existing Alberta guidelines to assess the quality of water drained from coal mine sites in the East Slope Foothills of Alberta. World literature was reviewed within the context of the east slopes environment and current mining operations. The ability of coal mine operators to meet the various guidelines is discussed.

** 32. RRTAC 87-6: Assessing Design Flows and Sediment Discharge on the Eastern Slopes. Hydrocon Engineering (Continental) Ltd. and Monenco Consultants Ltd. 97 pp.

DESCRIPTION: The report provides an evaluation of current methodologies used to determine sediment yields due to rainfall events in well-defined areas. Models are available in Alberta to evaluate water and sediment discharge in a post-mining situation. SEDIMOT II (Sedimentology Disturbed Modelling Techniques) is a single storm model that was developed specifically for the design of sediment control structures in watersheds disturbed by surface mining and is well suited to Alberta conditions.

* 33. RRTAC 87-7: The Use of Bottom Ash as an Amendment to Sodic Spoil. S. Fullerton. 83 pp.

DESCRIPTION: The report details the use of bottom ash as an amendment to sodic coal mine spoil. Several rates and methods of application of bottom ash to sodic spoil were tested to determine which was the best at reducing the effects of excess sodium and promoting crop growth. Field trials

were set up near the Vesta mine in East Central Alberta using ash readily available from nearby coal-fired thermal generating station. The research indicated that bottom ash incorporated to a depth of 30 cm using a subsoiler provided the best results.

* 34. RRTAC 87-8: Waste Dump Design for Erosion Control. R.G. Chopiuk and S.E. Thornton. 45 pp.

DESCRIPTION: This report describes a study to evaluate the influence of erosion from reclaimed waste dumps on downslope environments such as streams and rivers. Sites were selected from coal mines in Alberta's mountains and foothills, and included resloped dumps of different configurations and ages, and having different vegetation covers. The study concluded that the average annual amount of surface erosion is minimal. As expected, erosion was greatest on slopes which were newly regraded. Slopes with dense grass cover showed no signs of erosion. Generally, the amount of erosion decreased with time, as a result of initial loss of fine particles, the formation of a weathered surface, and increased vegetative cover.

** 35. RRTAC 87-9: Hydrogeology and Groundwater Chemistry of the Battle River Mining Area. M.R. Trudell, R.L. Faught and S.R. Moran. 97 pp.

DESCRIPTION: This report describes the premining geologic conditions in the Battle River coal mining area including the geology as well as the groundwater flow patterns, and the groundwater quality of a sequence of several water-bearing formations extending from the surface to a depth of about 100 metres.

** 36. RRTAC 87-10: Soil Survey of the Plains Hydrology and Reclamation Project - Battle River Project Area. T.M. Macyk and A.H. MacLean. 62 pp. plus maps.

DESCRIPTION: The report evaluates the capability of post-mining landscapes and assesses the changes in capability as a result of mining, in the Battle River mining area. Detailed soils information is provided in the report for lands

adjacent to areas already mined as well as for lands that are destined to be mined. Characterization of the reconstructed soils in the reclaimed areas is also provided. Data were collected from 1979 to 1985. A series of maps supplement the report.

** 37. RRTAC 87-11: Geology of the Highvale Study Site: Plains Hydrology and Reclamation Project. A. Maslowski-Schutze. 78 pp.

DESCRIPTION: The report is one of a series that describes the geology, soils and groundwater conditions at the Highvale Coal Mine study site. The purpose of the study was to establish a summary of site geology to a level of detail necessary to provide a framework for studies of hydrogeology and reclamation.

** 38. RRTAC 87-12: Premining Groundwater Conditions at the Highvale Site. M.R. Trudell and R. Faught. 83 pp.

DESCRIPTION: This report presents a detailed discussion of the premining flow patterns, hydraulic properties, and isotopic and hydrochemical characteristics of five layers within the Paskapoo Geological Formation, the underlying sandstone beds of the Upper Horseshoe Canyon Formation, and the surficial glacial drift.

* 39. RRTAC 87-13: An Agricultural Capability Rating System for Reconstructed Soils. T.M. Macyk. 27 pp.

DESCRIPTION: This report provides the rationale and a system for assessing the agricultural capability of reconstructed soils. Data on the properties of the soils used in this report are provided in RRTAC 85-2.

** 40. RRTAC 88-1: Eccles, T.R., R.E. Salter and J.E. Green. A Proposed Evaluation System for Wildlife Habitat Reclamation in the Mountains and Foothills Biomes of Alberta: Proposed Methodology and Assessment Handbook. 101 pp. plus appendix.

DESCRIPTION: The report focuses on the development of guidelines and procedures for the assessment of reclaimed wildlife habitat in the Mountains and Foothills regions of Alberta. The technical section provides background documentation including a discussion of reclamation planning,

a listing of reclamation habitats and associated key wildlife species, conditions required for development, recommended revegetation species, suitable reclamation techniques, a description of the recommended assessment techniques and a glossary of basic terminology. The assessment handbook section contains basic information necessary for evaluating wildlife habitat reclamation, including assessment scoresheets for 15 different reclamation habitats, standard methodologies for measuring habitat variables used as assessment criteria, and minimum requirements for certification. This handbook is intended as a field manual that could potentially be used by site operators and reclamation officers.

** 41. RRTAC 88-2: Plains Hydrology and Reclamation Project: Spoil Groundwater Chemistry and its Impacts on Surface Water. M.R. Trudell (Compiler). Alberta Land Conservation and Reclamation Council Report #RRTAC 88-2. 135 pp.

DESCRIPTION: Two reports comprise this volume. The first "Chemistry of Groundwater in Mine Spoil, Central Alberta," describes the chemical make-up of spoil groundwater at four mines in the Plains of Alberta. It explains the nature and magnitude of changes in groundwater chemistry following mining and reclamation.
The second report, "Impacts of Surface Mining on Chemical Quality of Streams in the Battle River Mining Area," describes the chemical quality of water in streams in the Battle River mining area, and the potential impact of groundwater discharge from surface mines on these streams.

Available from: Publication Services
Queen's Printer
11510 Kingsway Avenue
Edmonton, Alberta T5G 2Y5

* A $5.00 fee is charged for handling and postage.
** A $10.00 fee is charged for handling and postage.
*** A $20.00 fee is charged for handling and postage.
N/A Not available for purchase but available for review at the Alberta Environment Library, 14th Floor, 9820-106 Street, Edmonton, Alberta T5K 2J6.

EXECUTIVE SUMMARY

The ability of actinorhizal shrubs to tolerate inhospitable conditions while improving soil fertility and organic matter status has led to increased usage of these plants for land reclamation and amenity planting purposes. Silver-berry and buffalo-berry are two such shrubs which are being tested as potential candidates for the revegetation of the oil sands tailings in northeastern Alberta.

Associated with the roots of silver-berry and buffalo-berry are two symbionts - the N_2-fixing actinomycete, Frankia, and the vesicular-arbuscular mycorrhizal (VAM) fungi. Numerous studies have demonstrated that, particularly in nutrient limited conditions, mycorrhization and nodulation can result in significantly better plant performance as a consequence of improved N and P nutrition. The benefits conferred on the host by the symbionts may assume even greater importance in the revegetation of mine tailings which are notoriously nutrient-poor.

In addition to reducing soil fertility, the upheaval and mixing of soil during the mining process can lower Frankia and VAM inoculum levels. Both soil fertility and symbiont inoculum potential can be improved by introducing an organic amendment to the minespoil. Soil reconstruction on the oil sands tailings is facilitated by the application of muskeg peat which is stockpiled on the site for reclamation purposes. Alternatively, if woody plants are raised as containerized seedlings, they can be inoculated with both their N_2-fixing and mycorrhizal symbionts prior to being outplanted. However, before embarking on a large-scale inoculation program which will ultimately raise the cost of producing a seedling, factors such as plant dependency on the symbionts, the level of Frankia and mycorrhizal inoculum in the outplanting soil and the nodule/mycorrhizal status of containerized seedlings leaving commercial greenhouses should be considered. With this in mind, a research program was initiated to fulfil the following objectives:

1. To determine the mycorrhizal affinities of various actinorhizal shrubs in the Fort McMurray, Alberta region.
2. To establish a basis for justifying symbiont inoculation of buffalo-berry and silver-berry. Factors investigated included: i) the dependency of the shrubs on their mycorrhizal and N_2-fixing symbionts as expressed in plant performance under inoculated and uninoculated conditions, ii) the Frankia and VAM inoculum levels in the outplanting soil including stockpiled peat on the Syncrude lease and soils reconstructed from peat, mineral soil and oil sand tailings; iii) rates of mycorrhization and nodulation in undisturbed and reconstructed tailings dyke soils, and iv) the mycorrhizal and nodule status of containerized shrubs raised in commercial and provincial nurseries.
3. To develop a growing regime for the greenhouse production of mycorrhizal, nodulated silver-berry and buffalo-berry.
4. To conduct a field trial on reconstructed soil on the Syncrude site to critically evaluate the growth performance of inoculated silver-berry and buffalo-berry as compared with their uninoculated counterparts.

The major findings are:

JUSTIFICATION FOR INOCULATION

1. In Alberta, silver-berry and buffalo-berry are strictly VA-mycorrhizal. Levels of VAM colonization in roots of field collected plants can be as high as 60% suggesting a high degree of symbiont dependency under field conditions.
2. Silver-berry and buffalo-berry are highly dependent on their symbionts for optimum growth as evidenced by four (silver-berry)and nine-fold (buffalo-berry) increases in shoot weights when seedlings are inoculated with Frankia and VA-mycorrhizal fungi.

3. The VAM inoculum potential of both stockpiled and undisturbed muskeg peat is negligible due to the absence of VAM hosts.
4. Due to the low levels of VAM inoculum in the peat, stockpiling has no significant impact on VAM propagule levels. Vegetating peat stockpiles with VAM hosts such as grasses and legumes can increase VAM infectivity by 10-12% over six years. Tailings sand amended with peat would lack VA-mycorrhizal inoculum unless the peat had been vegetated with VAM hosts for a substantial length of time.
5. Soil from mixed woodlands (spruce, aspen, pine) has the highest VAM and Frankia inoculum potential of all soils assayed in the Ft. McMurray area. Amendation of tailing sand with this type of soil would greatly improve symbiont infectivity.
6. Growth of slender wheatgrass in unfertilized, stockpiled peat is stimulated when inoculated with mycorrhizal fungi, suggesting VAM fungi are necessary to satisfy the nutritional demands of the plant when grown in P-deficient peat.
7. Containerized shrubs grown in various nurseries in Alberta and B.C. are seldom mycorrhizal and/or nodulated if less than one year old. This means the majority of actinorhizal shrubs are symbiont-free if shipped to the buyer within a year of planting. Containerized shrubs which are more than one year old and have spent time in the shadehouse may or may not be colonized by their symbionts.
8. Buffalo-berry planted in reconstructed soil in the greenhouse do not become mycorrhizal or nodulated until eight weeks after planting. Since rates of colonization would be expected to be much slower in the field than in the

greenhouse and since the growing season in the oil sands region is short, it is doubtful that containerized shrubs would obtain much benefit from the symbiosis during the first growing season unless artificially inoculated.

9. Uninoculated silver-berry seedlings outplanted on the Suncor dyke exhibited relatively rapid mycorrhization (within six weeks of planting) presumably due to high VAM inoculum levels resulting from the predominance of VAM hosts (grasses, legumes) on the dyke. In contrast, nodulation was poor caused by either a lack of Frankia inoculum in the soil or poor root growth out of the planting plug.
10. The low VAM/Frankia inoculum potential and the slow rates of mycorrhization and nodulation in reconstructed soil on the tailings sand dykes, combined with the high dependency of silver-berry and buffalo-berry on their symbionts, forms a strong basis for artificial inoculation of containerized seedlings.

GROWING REGIME FOR PRODUCING MYCORRHIZAL, NODULATED SEEDLINGS

11. In order to produce mycorrhizal, nodulated silver-berry and buffalo-berry seedlings of suitable size and quality, fertilization should not exceed 56 mg N and 12 mg P per application. Fertilizer concentrations in excess of this do not totally eliminate symbiont colonization, but mycorrhizal and nodule development is severely reduced. The optimum fertilization regime in this study was 200 mg L^{-1} 28-14-14 Plant Prod Soilless Feed applied twice weekly.
12. Silver-berry growth is significantly better in 150 cc containers than in 65 cc containers. As soil volume is reduced there is a concomitant decrease in symbiont growth response so that inoculated seedlings in the 150 cc containers exhibited a significant growth response whereas those in 65 cc containers did not.

13. Seedlings inoculated with woodland soil demonstrate better mycorrhization and nodulation and a greater growth response at 26°C than at 16°C.
14. In a Frankia inoculum trial, the best sources of inoculum resulting in the biggest silver-berry seedlings with the most heavily nodulated root systems were found to be wild buffalo-berry soil, crushed silver-berry nodules, and crushed silver-berry nodules treated with polyvinyl pyrrolidine to reduce oxidation of phenols which inhibit Frankia growth. Seedlings inoculated with Frankia pure culture obtained from Rhizotec Labs in Quebec became heavily nodulated but this was not manifested in improved plant growth. Seedlings inoculated with a pure culture of Frankia isolated from buffalo-berry failed to become nodulated possibly because the Frankia strain was incompatible with silver-berry.
15. Mixing a highly infective soil into the planting mixture appears to be more effective at promoting symbiont development than applying the inoculum as a soil slurry after plant establishment.
16. Mycorrhizal, nodulated silver-berry and buffalo-berry of suitable size and quality can be obtained by planting them in 150 cc containers filled with peat/vermiculite (1/1 v/v) which has been supplemented with high inoculum soil (10-15% by volume), and fertilizing them twice weekly at a rate of 200 mg L^{-1} 28-14-14 Plant Prod Soilless Feed. Buffalo-berry appears to be more N-demanding than silver-berry and may require a higher fertilizer concentration.

FIELD TRIAL TO TEST GROWTH RESPONSE OF INOCULATED SILVERBERRY AND BUFFALO-BERRY

17. Overwinter mortality was higher for inoculated shrubs than for uninoculated shrubs. Due to their symbiotic condition, the inoculated shrubs may have had greater stomatal conductance and higher rates of transpiration than the uninoculated shrubs when outplanted, making them more susceptible to frost damage. It is possible that inoculated seedlings require a longer period of hardening off than do uninoculated seedlings, particularly if they are to be outplanted in the fall.
18. After one growing season, shoot weights of inoculated silver-berry were three to seven times greater than those of the uninoculated seedlings, while shoots of inoculated buffalo-berry were three to five times heavier than those of their uninoculated counterparts. The much superior growth performance of the inoculated seedlings was continued over the second growing season.
19. The significant growth response of the inoculated shrubs aboveground was reflected in the symbiont status below-ground where nodule and mycorrhizal development was significantly more extensive in the inoculated plants than the uninoculated plants over the two growing seasons.
20. Shoot production appeared to be heavily dependent on healthy nodule development as evidenced by the highly significant correlations between shoot weights and nodule weights ($r = 0.91, 0.97$) after one growing season. Shoot productivity was more closely related to nodule status than mycorrhizal status ($r = 0.63$ and 0.58 for shoot weight versus mycorrhizal root length for silver-berry and buffaloberry, respectively). Also, per cent mycorrhizal root length was not closely correlated with nodule number and weight suggesting that, in the field, other factors besides mycorrhizal status may strongly influence nodulation.

21. The much superior growth performance of inoculated seedlings compared with uninoculated seedlings over two growing seasons provides unequivocal proof that pre-inoculation with mycorrhizal and N_2-fixing symbionts can, in the case of silver-berry and buffalo-berry, result in more rapid revegetation of oil sands tailings. It is strongly recommended that containerized silver-berry and buffalo-berry seedlings, destined for reclamation and possibly forestry sites, be inoculated with Frankia and mycorrhizal fungi prior to outplanting.

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ACKNOWLEDGEMENTS

This research was funded by the Reclamation Research Technical Advisory Committee of the Alberta Land Conservation and Reclamation Council, using Heritage Savings Trust Fund monies. The cooperation of Syncrude Canada Ltd. and Suncor Inc. for providing planting sites and aid in plot establishment is gratefully acknowledged. In particular, we would like to thank Tony Dai and Kim McCumber of Syncrude Canada Ltd. for their help and advice during the field work. The support and enthusiasm for this study demonstrated by Chris Powter and Jim Campbell of the Research Management Division is very much appreciated. The authors thank the following for providing nursery stock: T. Laidlaw (Laidlaw Vegetation Consulting Ltd.), B. Hutchinson (Whitecourt Mountain Seedling Nursery), G. Granger (Alberta Tree Nursery and Horticulture Centre), C. Jones (Reid, Collins Nurseries Ltd.), and B. Novlesky (Syncrude Canada Ltd.). Silver-berry and buffalo-berry seeds were provided by the Alberta Forest Service. In conclusion, this study would not have been possible without the invaluable technical assistance provided by L. Burton, P. Mazier, C. McBain and C. Anderson and the statistical analyses provided by C. Griffiths. We are grateful to Lynn Ewing and Della Patton for their help in typing and organizing the report.

1. INTRODUCTION

Actinorhizal plants are non-leguminous, N_2-fixing plants whose nodules are formed by the actinomycete, Frankia, rather than by the bacterium, Rhizobium, as is the case for legumes. They are perennial, woody trees or shrubs which often colonize nutrient-poor, marginal or disturbed habitats such as sand dunes, wet bogs, dry sandy or gravelly areas and mine wastes (Torrey, 1978). They have the ability to fix up to 300 kg atmospheric nitrogen ha^{-1} $year^{-1}$ and, consequently, are seriously being considered as an alternative to nitrogen fertilizer as a management tool for intensive forestry in Canada (Fortin et al., 1984). Alder, in particular, has been shown to significantly improve the nitrogen status of forest soils (Cot@ and Camir@, 1985; Huss-Danell, 1986; Malcolm et al., 1985; Tarrant and Trappe, 1971; Wheeler et al., 1986) and minespoils (Heilman and Ekuan, 1982; Tarrant and Trappe, 1971), but this has (Cot@ and Camir@, 1985; DeBell and Radwan, 1979; Hansen and Dawson, 1982) or has not (Heilman and Ekuan, 1982; Malcolm et al., 1985) been manifested in improved productivity of commercial tree species in mixed plantings. In addition to improving soil nitrogen levels, actinorhizal plants ameliorate soil temperature and moisture conditions through the accumulation of organic matter resulting from leaf and root litter deposition and decomposition. The ability of actinorhizal shrubs to tolerate inhospitable conditions while improving soil fertility and organic matter status has led to increased usage of these plants for land reclamation and amenity planting purposes (Fessenden, 1979).

The actinorhizal plants which are native to Alberta include green alder (Alnus crispa (Ait.) Pursh), river alder (A. tenuifolia Nutt.), snow brush (Ceanothus velutinus Dougl. ex Hook.), silver-berry (Elaeagnus commutata Bernh. ex Rydb.), buffalo-berry (Shepherdia canadensis (L.) Nutt.) and the yellow and white dryads (Dryas drummondii Richards., D. octopetala ssp. hookeriana [Juz.] Hult.). Of these, green alder, silver-berry and buffalo-berry are being tested as potential candidates for the revegetation of oil sands tailings resulting from the extraction of oil from the oil sand deposits located in northeastern Alberta. This report is concerned exclusively with silver-berry and buffalo-berry.

In addition to having the N_2-fixing symbiont associated with their roots, both silver-berry and buffalo-berry form mycorrhizae -- the mutual symbiosis between specific fungi, in this case vesicular-arbuscular mycorrhizal (VAM) fungi, and the plant root. The fungus improves the phosphorus nutrition of the plant by exploring a greater volume of soil for the relatively immobile PO_4 ion than the plant root itself would be capable of doing, while the fungus benefits by receiving carbohydrates from the plant. The potential importance of VA mycorrhizae in enhancing the revegetation of minespoils has concentrated primarily on forage and crop species (Khan, 1981; Lambert and Cole, 1980; Zak and Parkinson, 1982, 1983) with woody shrubs receiving much less attention. However, due to the coarse-rooted nature of many woody shrubs, it is possible that these species are more dependent on the VA mycorrhizal symbiosis than fibrous-rooted species where soil-root contact is high (Hayman, 1982). This would explain the significant growth enhancement observed in many woody species inoculated with a wide variety of VAM fungi (Furlan et al., 1983, Kormanik et al., 1982; Plenchette et al., 1981; Pope et al., 1983) and the much improved growth of VAM-inoculated rabbit brush and fourwing saltbush in coal minespoil (Aldon, 1978; Lindsey et al., 1977).

Numerous studies, designed to clarify the interactions between VAM fungi and Rhizobium in legumes, have shown that mycorrhizal infection can significantly stimulate nodulation, nitrogenase activity and in some cases foliage N concentrations (Ames and Bethlenfalvay, 1987; Azcon-Aguilar and Barea, 1981; Barea et al., 1980; Barea and Azcon-Aguilar, 1983; Carling et al., 1978; Ganry et al., 1982; Green et al., 1983; Redente and Reeves, 1981; Smith and Daft, 1977; Smith et al, 1979). It has been demonstrated that both nodule initiation and N_2-fixation have a high P requirement which is satisfied by the mycorrhizae resulting in significantly greater plant productivity (Smith et al., 1979). With the exception of Rose and Youngberg (1981), who observed that the actinorhizal shrub, Ceanothus velutinus, exhibited greater shoot and root weights, greater number and weight of nodules and more nitrogenase activity if colonized by both the mycorrhizal fungi and Frankia than if colonized by Frankia alone, research elucidating the dependence of actinorhizal plants on both symbionts has been

lacking. Considering the potential importance of both the VAM fungi and Frankia in the establishment, survival and growth of actinorhizal shrubs on marginal and disturbed habitats such as mine tailings, it is surprising that so little information is available on the role of these symbionts in accelerating the revegetation process.

The value of actinorhizal shrubs for improving the fertility and organic matter status of soils which are prone to significant losses of N as a result of intensive forestry practices, has led researchers in Quebec to develop a program for isolating, characterizing and evaluating the effectiveness of Frankia strains from green alder (Normand et al., 1984) with the final goal being large scale inoculation of alder on a commercial basis (Périnet et al., 1985). Subsequent field trials with inoculated and uninoculated alders demonstrated that, over the long term (3 years), inoculation with an effective strain of Frankia significantly improved growth of three species of alder (Burgess et al., 1986). Inoculation with mycorrhizal fungi was not addressed in these studies.

In Alberta the establishment of woody trees and shrubs on disturbed sites is usually accomplished by outplanting containerized seedlings which have been raised and hardened off in commercial or provincial greenhouse operations. The use of containerized seedlings offers a good opportunity for introducing N_2-fixing and mycorrhizal symbionts to the plants prior to being outplanted.

However, before embarking on a large-scale inoculation program which will ultimately raise the cost of producing a seedling, a number of factors should be considered. These include the degree of dependency of a plant on its symbionts, the level of Frankia and mycorrhizal inoculum in various soils into which the seedlings will be outplanted and the effectivity of this inoculum, the nodule/mycorrhizal status of containerized seedlings leaving commercial greenhouses, and the rates of nodulation and mycorrhizal colonization of seedlings once outplanted. Prior consideration of these factors will determine whether or not the time and effort required to develop an inoculation program is worthwhile. Once the decision is made to enter into an inoculation program, it becomes necessary to develop a growing regime for rearing mycorrhizal, nodulated seedlings of an acceptable size. For this the

optimum nutrient conditions (fertilizer rates), soil pH, light conditions, container volume, soil temperature, Frankia and mycorrhizal inoculum source and method of inoculation should be evaluated. Finally, it is essential that field trials be conducted to establish unequivocally that inoculated seedlings will outperform uninoculated seedlings over the long-term under field conditions. On the basis of the foregoing discussion, research was conducted to fulfil the following objectives.

1.1 OBJECTIVES

1. To determine definitively the mycorrhizal affinities of various actinorhizal and other woody shrubs in the Fort McMurray, Alberta region.

2. To establish a basis for promoting the symbiont inoculation of two actinorhizal shrubs, buffalo-berry and silver-berry. Factors which were investigated include:
   i) the dependency of the shrubs on their mycorrhizal and N_2-fixing symbionts as expressed in plant performance under inoculated and uninoculated conditions.
   ii) the Frankia and VAM inoculum levels in the soil into which the shrubs would be planted, i.e. peat stockpiled on the Syncrude lease, soils reconstructed from peat, mineral soil and oil sand tailings on the tailings dykes and woody species trial plots on the Syncrude lease.
   iii) the mycorrhizal and nodule status of containerized shrubs raised in commercial and provincial greenhouses.
   iv) rates of mycorrhization and nodulation in undisturbed and reconstructed tailings dyke soils.

3. To develop a growing regime for the greenhouse production of mycorrhizal, nodulated silver-berry and buffalo-berry.

There are basically two approaches to the development of an inoculation program. The first, termed the "high tech" approach, involves the isolation of species or strains of VA mycorrhizal fungi and N_2-fixing Frankia into pure culture, propagating the isolates and then using pure culture inoculum to inoculate containerized seedlings. The second, the "low tech" approach, involves mixing field soil with a high symbiont inoculum potential into the planting mixture prior to filling and planting the containers. Due to time constraints the second approach was investigated in this study. This entailed testing such factors as fertilization regimes, container volume, soil temperatures, inoculum sources, and time and method of inoculation.

4. To conduct a field trial on reconstructed soil on the Syncrude site to critically evaluate the growth performance of inoculated silver-berry and buffalo-berry as compared with their uninoculated counterparts.

2. STUDY AREA

The study area was located in the Athabasca Oil Sands region near Fort McMurray in northeastern Alberta (Figure 1). This region is situated within the Mixedwood Section of the Boreal Forest Region (Rowe, 1972) and has a gently undulating topography with sandy soils dominating the upland areas and wet peatland occurring in the poorly drained areas. The vegetation consists predominantly of white spruce and aspen forest with jack pine-lichen woodlands occurring on the sandy upland areas and black spruce/tamarack bogs in the poorly drained, low lying areas. The climate is cool continental characterized by relatively short, cool summers and long cold winters. Mean annual temperature and precipitation in the Fort McMurray area are -0.2°C and 472 mm, respectively. Gray luvisolic soils are characteristic of the aspen-white spruce mixedwood forests, while eluviated dystric brunisols predominate in the jack pine-lichen woodlands. More detailed information on the climate, vegetation and soils of the region can be obtained from Strong and Leggat (1981) and Turchenek and Lindsay (1982).

Soils for greenhouse growth studies and VA mycorrhizal and Frankia inoculum screening were sampled mainly from aspen-white spruce mixed woodland in close vicinity to the Mildred Lake Research Facility (Figure 1) and from reconstructed soil on the tailings dyke located on the Syncrude Oil Sands Lease. Samples to determine the effect of stockpiling muskeg peat on VA mycorrhizal inoculum were removed from an eastern larch/black spruce/Labrador tea/moss peat bog and from the NT-2 peat stockpile, both located on the Syncrude Lease. The stockpile was 300 m wide and 3 m deep and was 8 months old when sampled. Rates of nodulation and mycorrhization of silver-berry were determined on seedlings outplanted on plots established on the Suncor Tar Island Dyke facing the Athabasca River on the Suncor Oil Sands Lease. The plots were established in areas which had been revegetated with grass/legume mixtures in 1971, 1974 and 1978.

The field trial to evaluate the growth response of inoculated silver-berry and buffalo-berry was conducted on two plots established adjacent to the RRTAC soil reconstruction-woody plant experimental area on the Syncrude Oil Sands Lease. The experimental area was located on a specially prepared pad of oil sand tailings which had been amended

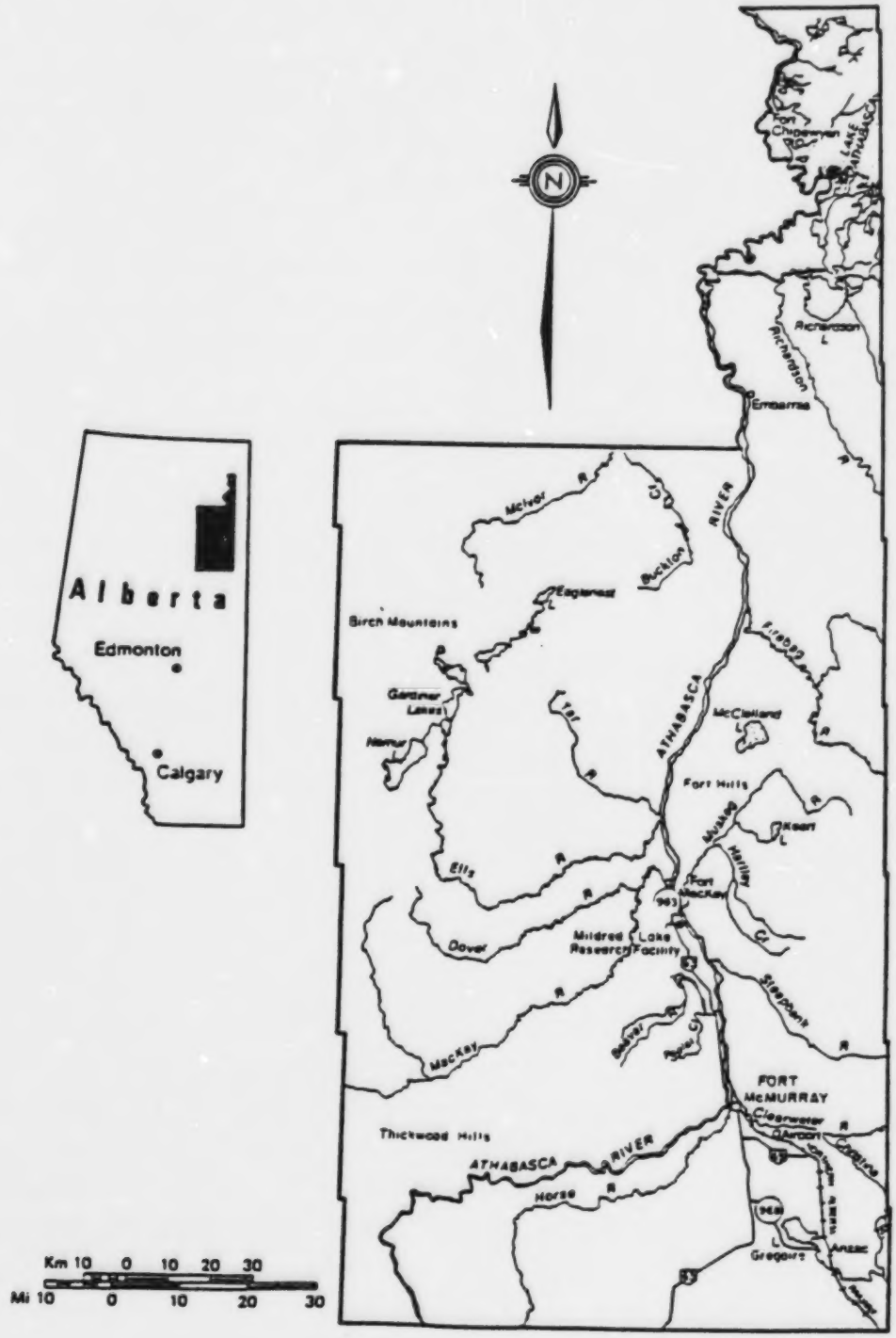

Figure 1. Map showing location of the study area (excerpted from Turchenek and Lindsay, 1982).

with various amounts of muskeg peat and surficial overburden clay. Further details regarding the plots are given in reports prepared by Hardy Associates (1983, 1984).

3. METHODS

3.1 MYCORRHIZAL STATUS OF WOODY SHRUBS

Although it is widely accepted that grasses and the majority of herbaceous plants are mycorrhizal with vesicular-arbuscular (VA) mycorrhizal fungi, the mycorrhizal affinities of woody shrubs, including actinorhizal shrubs, are less well-known. In order to establish the mycorrhizal condition of silver-berry, buffalo-berry and various other shrubs growing in the wild, the following survey was conducted.

Buffalo-berry and silver-berry plants were sampled from the Vaartnou reclamation plots near the Mildred Lake campsite, from a cutbank near the Suncor plant and from a roadcut near the University of Calgary Research Station in the Kananaskis Valley. In addition, saskatoon-berry and cinquefoil were sampled from a cutline near the Mildred Lake campsite and the Vaartnou plots, respectively. Five replicate plants were excavated at each sampling location, the roots were washed, and only those roots which were attached to the stem of the host species in question were assessed for mycorrhizal development. The root systems were scanned under a dissecting microscope for ecto-mycorrhizal development and subsamples subsequently cleared and stained for the detection of VA mycorrhizae (Phillips and Hayman, 1970).

3.2 JUSTIFICATION FOR INOCULATION OF CONTAINERIZED SILVER-BERRY AND BUFFALO-BERRY

Before undertaking a widescale inoculation program, a number of factors regarding the necessity of such a program should be considered. In this study the factors which were investigated include:

i) the dependency of the shrubs on their N_2-fixing and mycorrhizal symbionts, i.e. how do the shrubs perform in the presence and absence of their symbionts and what benefits are conferred on the host by the symbionts Woody species vary in their symbiont dependency, and it could be argued that if they are not highly dependent (based on stimulation of root and shoot production in the presence of the symbionts) there is less of a need to inoculate them prior to outplanting.

ii) symbiont inoculum levels in the soil into which the shrubs will be planted. The symbiont inoculum potential is determined primarily by the degree of disturbance of a soil and by the host plant species present in a particular site. For example, massive soil upheaval characteristic of most mining operations can reduce symbiont inoculum potential substantially, while the dominance of non-host plant species, i.e. those which are neither N_2-fixing or VA mycorrhizal, can also lead to a reduction in symbiont inoculum. Shrubs would be expected to benefit most from pre-planting inoculation when planted into soil lacking symbiont inoculum.

iii) VA mycorrhizal and N_2-fixing nodule status of containerized shrubs prior to outplanting. While being raised in the greenhouse, containerized plants may become colonized by their symbionts via inoculum in the planting mixture, the water or the atmosphere. If this is the case, artificial inoculation may be unnecessary.

iv) rates of mycorrhizal colonization and nodulation. Rapid colonization by the symbionts after outplanting will ensure that the host plants will derive maximum benefit from the mycorrhizal/N_2-fixing relationship. If colonization is slow, however, inoculation may be necessary to accelerate plant establishment and growth. This would apply, particularly, in northern regions where the short growing season could reduce symbiont colonization rates to such a degree that the plant would not begin to benefit from its symbionts until the end of the growing season. The various experiments which were designed to elucidate the preceding factors follow.

3.2.1 The Dependency of Silver-berry and Buffalo-berry on their Mycorrhizal and N_2-Fixing Symbionts

Oil sand tailings and peat from the Syncrude NT2 stockpile were mixed based on 11 and 5.5 cm depth equivalents for the sand and peat, respectively. A 3.5 cm depth equivalent of forest soil collected from beneath buffalo-berry shrubs growing in an aspen stand near Mildred Lake and containing a high symbiont inoculum potential was added to half of the oil sands/peat mixture. This represented the inoculated treatment. The uninoculated treatment was identical to the

inoculated treatment with the exception that the buffalo-berry inoculum was autoclaved to eradicate the symbionts. Sections of sewer pipe, 20 cm deep and 7.7 cm diameter, the bottoms of which were covered with a layer of polyester batting and a piece of fiberglass screening, were filled with soil mixtures from each treatment.

Buffalo-berry seeds were scarified in concentrated H_2SO_4 for 30 min (King, 1980), rinsed in cold running water overnight and germinated on moist filter paper. Silver-berry seeds were leached in cold, running water for 4 days and germinated on moist filter paper (King et al., 1983). One germinant of each species was planted in each of 10 replicate cores (total = 40 cores) and the cores placed in the greenhouse in December in a random arrangement. Light intensity on clear days was 500 $\mu Em^{-2}sec^{-1}$ (198 W m^{-2}, 26 klx), 156 $\mu Em^{-2}sec^{-1}$ (67 W m^{-2}, 9 klx) on cloudy days and day length was extended to 20 hours with a minimum of 74 $\mu Em^{-2}sec^{-1}$ (20 W m^{-2}, 3.5 klx). Temperatures were generally between 18 and 25°C, but occasionally fell to 5°C at night, and were 30°C during the day. Plants were watered twice weekly without any additions of nutrients. All seedlings were harvested after 12 weeks.

Shoots were removed, dried at 80°C and weighed. Roots were separated from the soil, washed and the nodules counted and weighed. Vesicular-arbuscular mycorrhizal assessments were determined on subsamples by the method of Zak and Parkinson (1982). Remaining roots were dried at 80°C and weighed.

3.2.2 Levels of Mycorrhizal Inoculum in Outplanting Soil

During the course of the last decade numerous soils collected from the Fort McMurray region have been assayed for their VA-mycorrhizal inoculum potential. Assays consisted of a baiting technique in which slender wheatgrass germinants were planted in soil or peat which had been well-mixed and packed into 65 or 150 cc Leach Cone-tainers. The plants were grown in the greenhouse and were not fertilized. After 8 to 12 weeks, the roots were separated from the soil, cleared, stained and assessed for mycorrhizal development using the methods given in 3.2.1. The various soils which were assayed are listed in Table 3 of the Results section.

3.2.3 Growth Characteristics and Mycorrhizal Potential of Undisturbed Bog Peat and Stockpiled Peat

Before mining the oil sands, the muskeg peat, which often overlies the oil-bearing sand, is drained, stripped and stockpiled for subsequent revegetation of the tailings sand dykes. Little is known of the VA mycorrhizal status of undisturbed peat and the impact of stockpiling on mycorrhizal propagules. The plants being used to revegetate the dykes (e.g. grasses, legumes) and those being considered for reclamation purposes (e.g. woody shrubs such as saskatoon-berry, buffaloberry, silver-berry, wild rose etc.) are VA mycorrhizal and may be heavily dependent on their mycorrhizal associates for both growth and survival. Therefore, it was considered essential that the mycorrhizal potential of both undisturbed and stockpiled peat be investigated.

Twenty-five peat samples were randomly removed from each of two depths (0-15 cm; 50-100 cm) in an undisturbed peat bog and a peat stockpile on the Syncrude site near Fort McMurray. The vegetation on the bog was predominantly eastern larch, swamp birch, Labrador tea and feather and sphagnum mosses while the peat stockpile, which was 8 months old, was largely unvegetated. Samples were removed every 10 m along a 250 m transect on each site.

Each sample was thoroughly mixed, packed into a 65 cc Leach Cone-tainer and planted with a slender wheatgrass germinant. Plants were grown in the greenhouse under the light conditions described in 3.1.1 and received no fertilizer. After 12 weeks, shoot weights, root weights, total root length and % VAM infection were determined using the methods described in 3.2.1.

3.2.4 Mycorrhizal Potential of Revegetated Dyke Peat

The University of Calgary experimental plot on the Syncrude dyke was reclaimed with peat from a 6 year old stockpile which had been revegetated with a grass mixture. Therefore, it was decided to sample this peat to determine if storage time and presence of a VA mycorrhizal host had changed the mycorrhizal inoculum potential.

Five peat samples (0-20 cm deep) were randomly removed from the northern boundary of the experimental plot, mixed and packed into 20, 65 cc Leach Cone-tainers, 4 per sample. Each Cone-tainer was

planted with a pre-germinated slender wheatgrass seedling. The seedlings were then divided into two treatments--ten seedlings received Plant Prod Soilless Feed at a rate of 100 mg L^{-1} 15-15-18 twice per week and sequestrene-Fe at 56 ppm twice per week while the remaining 10 seedlings received only deionized water. Plants were grown in the greenhouse under a 20h daylength, with a minimum of 3.5 klx light intensity. The parameters measured after 9 weeks included shoot and root weights, root length and % VAM colonization using the methods described in 3.2.1.

3.2.5 Effect of VA Mycorrhizal Inoculation on Plant Performance of Slender Wheatgrass Grown in Stockpiled Peat under Fertilized and Unfertilized Conditions

Preliminary examination of plants grown in peat stockpiled for 8 months revealed that there was a paucity of VAM inoculum in this peat. Therefore, an experiment was conducted to determine if the addition of VA inoculum would improve the growth of slender wheatgrass under fertilized or unfertilized conditions.

Peat from the 50-100 cm depth of the 8 month old NT-2 stockpile was bulked and separated into two batches. One batch was mixed 50/50 (v/v) with root/sand inoculum from a Glomus aggregatum (a common VA fungus having a wide distribution in Alberta) pot culture while the other batch was mixed 50/50 (v/v) with autoclaved root/sand inoculum to serve as a control. Twenty Leach Cone-tainers were packed from each batch and one pre-germinated slender wheatgrass seedling was planted in each container. Seedlings were grown in the greenhouse under the conditions outlined in 3.2.4. Five seedlings from each treatment (inoculated, fertilized; uninoculated, fertilized; inoculated, unfertilized; uninoculated, unfertilized) were sampled when the plants were 4 and 10 weeks old. Shoot and root weights and VA mycorrhizal development were assessed as described previously.

3.2.6 Mycorrhizal and Nodule Status of Containerized Shrubs Planted on the Oil Sands Tailings Reconstruction Plots

If shrub species which are highly dependent on their symbionts for growth and survival are used for reclamation purposes, both the

symbiont inoculum potential in the reconstructed soil and the symbiont status of the shrubs prior to outplanting should be determined. Consequently, it was decided that the mycorrhizal and nodulation status of the shrubs outplanted on the RRTAC oil sands tailings reconstruction plots (pad plots) in the fall of 1984 should be assessed.

Wild rose, pin cherry, saskatoon-berry, Canada buffalo-berry and silver-berry, grown in either the Syncrude or Laidlaw nurseries, were sampled in August. Twenty-five plants of each species were randomly selected to determine plant weights and mycorrhizal and nodule status. All plants had been grown in 150 cc Spencer-Lemaire Hillson book containers. Roots were washed free of soil, examined for nodules and then cleared, stained and examined for VA mycorrhizae (3.2.1). Shoot and root weights were determined after drying at 80C.

3.2.7 Growth Characteristics and Symbiont Status of Woody Shrubs Raised in Various Commercial Nurseries in Alberta and British Columbia

As mentioned previously, containerized shrubs can become mycorrhizal or nodulated during the growing and hardening-off phases in the greenhouse and shadehouse via inoculum in the planting mixture, water or atmosphere. If seedlings become heavily mycorrhizal or nodulated prior to outplanting, artificial inoculation may not be necessary. Since little is known of the symbiont status of shrub species grown in commercial greenhouses, a survey of various nursery-grown shrubs was conducted.

Nine species of woody shrubs were sampled from the Whitecourt, Laidlaw, Oliver and Syncrude nurseries in Alberta and the Reid-Collins nursery in British Columbia in August, 1985. Names of the shrubs, crop year, container size and number of seedlings assayed are detailed in Table 8 of the Results. Shoot heights were measured for some of the species, while shoot and root weights were determined for all plants after drying at 80C. The N_2-fixing shrubs were assessed for nodule numbers and weights. Vesicular-arbuscular mycorrhizal status of all the shrubs was determined by clearing, staining and examining under a dissecting microscope, a 10% subsample of the total wet weight of each root system.

3.2.8 Rates of Mycorrhization and Nodulation in Buffalo-berry Grown in Woodland Soil and Amended Tailings Sand in the Greenhouse

The rate of mycorrhizal and nodule development from indigenous soil inoculum may determine to a large degree the benefits derived by the plant from its symbionts during the first growing season after outplanting. This applies particularly to the oil sands region where the short growing season and potentially low symbiont inoculum levels in the reconstructed soil on the tailings dykes may result in such slow colonization rates that the plants do not benefit from their symbionts until the end of the growing season. Therefore the rates of infection may determine whether or not pre-planting inoculation is necessary. With this in mind a study was conducted to determine the rates of mycorrhizal and nodule development of buffalo-berry planted in undisturbed woodland soil and amended tailings sand.

Five soil samples were removed from the forest floor of a mixed woodland (poplar, spruce, buffalo-berry, alder) near the Mildred Lake campsite. The roots were coarsely chopped, the samples were bulked and the soil/root mixture packed into 25, 150 cc Leach Cone-tainers. The procedure was repeated with 0-15 cm deep reconstructed soil (tailings sand, 3% peat, 12% clay) removed from the University of Calgary soil reconstruction plots adjacent to the RRTAC soil reconstruction site. Buffalo-berry seed was stratified and germinated as described previously (3.2.1) and one germinant planted in each container. Plants were grown in the greenhouse under the conditions outlined in 3.2.1 and were watered with deionized water when required. At 2, 4, 8, 12, and 20 weeks after planting, 5 seedlings were destructively sampled and shoot and root weights, % mycorrhizal colonization and nodule weights measured using the methods described in 3.2.1.

3.2.9 Rates of Mycorrhizal and Nodule Development in Silver-berry Outplanted in an Undisturbed Woodland and the Suncor Tar Island Dyke

This study was conducted to gain some insight into the rates of symbiont colonization under field conditions.

Silver-berry seed was stratified and germinated using the technique described in 3.2.1. Germinants were planted in autoclaved

peat/vermiculite (50/50, v/v) in 150 cc containers. Plants were grown in the greenhouse for 2 months using the light conditions described in 3.2.1. Seedlings were fertilized with 200 mg 28-14-14 twice weekly and flushed with deionized water between fertilizer applications. Prior to outplanting, seedlings were hardened off outdoors for 2 weeks without any fertilization.

Three 10m x 10m plots were established on the Suncor Tar Island Dyke in areas which had been revegetated with grass/legume mixtures in 1971, 1974 and 1978. Fertilization of the plots had been discontinued in 1979. Seedlings were planted in June, 1985. In each plot, 50 seedlings were planted at 1m intervals in 5 rows. The rows were 2m apart, 10 seedlings per row. The procedure was repeated in one additional plot established in an undisturbed mixed woodland (poplar, pine, spruce, wild rose, grasses etc.) located near the Mildred Lake campsite.

At 6 and 12 weeks after planting, 10 seedlings were randomly sampled from each plot, 2 seedlings per row. Shoot and root weights, root growth out of the planting plug, nodule weights and mycorrhizal development were measured for each plant using the methods described in 3.2.1. Survival was measured at the 6 week sample time.

3.3 DEVELOPMENT OF A GROWING REGIME FOR GREENHOUSE PRODUCTION OF MYCORRHIZAL, NODULATED SILVER-BERRY AND BUFFALO-BERRY

If, based on the factors discussed in 3.2, actinorhizal seedlings would benefit significantly from being colonized by their symbionts prior to being outplanted, it may become necessary to develop a program for inoculating containerized plants in the greenhouse or the shadehouse. There are many factors which are important in achieving successful inoculation; those investigated in this research program included fertilizer regimes, growing temperatures, container volume, inoculum source, and time and method of inoculation. Details of the various experiments follow.

3.3.1 Fertilizer Effects on Growth, Nodulation and Mycorrhizal Development in Buffalo-berry and Silver-berry

Ten random forest floor samples were removed from a mixed woodland (aspen poplar, white spruce, wild rose, buffalo-berry, grasses, herbs) located near the Mildred Lake campsite. An additional 10 samples were removed from plot 2 (tailings sand amended with 11 cm (3% organic C) muskeg peat and 2.9 cm (12% clay) surficial overburden clay) in the University of Calgary reclamation site (pad plot study). Each sample was subsampled and the subsamples for each soil type bulked. Forty containers were packed for each soil type and planted with either silver-berry or buffalo-berry which had been scarified and germinated as described previously. Five replicates of each species in each soil type were subjected to one of the following four fertilizer regimes: no fertilizer, 100 mg L^{-1}, 200 mg L^{-1} and 400 mg L^{-1} 28-14-14 Plant Prod Soilless Feed. Fertilizer applications were made twice weekly and plants were flushed with deionized water between applications to remove excess fertilizer salts. Plants were raised in the greenhouse in conditions similar to those described in 3.2.4.

After 20 weeks shoot and root weights after drying at 80°C, nodule wet weight and % root length colonized by mycorrhizal fungi were determined for each seedling using the methods described previously.

3.3.2 Effect of Container Volume and Inoculation on Growth of Silver-berry

Silver-berry seed was germinated as described in 3.2.1. Germinants were planted in 65 cc and 150 cc Cone-tainers (Ray Leach, Canby, OR) filled with 50/50 (v/v) autoclaved (sterilized) peat/vermiculite growing medium treated with autoclaved (control treatment) or unautoclaved VAM inoculum (inoculated treatment). The inoculum was collected from beneath buffalo-berry shrubs in a mixed woodland near the Mildred Lake campsite and was added to the planting mixture at a rate of 10% by volume. The seedlings were grown in the greenhouse (daylength extended to 20h with Gro-lux lights; minimum light intensity 3.5 klx) and received 200 mg L^{-1} 15-15-18 Plant Prod Soilless Feed once weekly during the first 4 weeks of growth and twice weekly for the remaining 16 weeks. Excess fertilizer was flushed out with deionized

water between fertilizer applications. There were 10 replicates for each of the uninoculated and inoculated treatments in each container size category.

Half of the seedlings in each treatment (i.e. 5 replicates) were harvested at 12 weeks and the remainder at 20 weeks. Shoot weight, root weight, nodule and mycorrhizal status were determined at each sample time. Mycorrhizal status was assessed by the methods of Phillips and Hayman (1970) and Zak and Parkinson (1982).

3.3.3 Growth of Silver-berry as Influenced by Soil Temperature and Symbiont Inoculation

Silver-berry was stratified and germinated as described previously (3.2.1). Leach Cone-tainers (150cc) were filled with soil mixtures belonging to each of the following four treatments:

i) uninoculated control - autoclaved peat/vermiculite (50/50, v/v) amended with autoclaved soil (20% by volume) from a Mildred Lake mixed woodland.

ii) woodland soil inoculum - as above with the exception that the woodland soil was not autoclaved.

iii) silver-berry soil inoculum - as above with the exception that soil inoculum originated from beneath silver-berry planted by Vaartnou in a reclamation plot near the Mildred Lake camp.

iv) VAM pot culture inoculum - as above but using *Glomus aggregatum* inoculum. *Glomus aggregatum*, a VAM fungus indigenous to Alberta, had been maintained in pure culture in the greenhouse on silver-berry planted in autoclaved peat/vermiculite. The inoculum consisted of pot culture soil and chopped silver-berry roots containing the fungus.

Half the seedlings were grown in a growth chamber programmed to maintain a constant temperature of 26°C in the root zone while the other half were placed in a chamber programmed to maintain a 16°C root zone. The experimental temperatures were based on soil temperatures measured in the University of Calgary greenhouse. These generally fell in the range of 20 to 30°C with extremes at 15° and 40°C. Day

length was set at 18 h and the air temperature was 24°C day/26°C night in the 26°C chamber and 11°C day/16°C night in the 16°C chamber. Light intensity 25 cm from the lights was 180 $\mu E\ m^{-2}\ sec^{-1}$ (170 $w\ m^{-2}$, 30 klx) in the 26°C chamber and 135 $\mu E\ m^{-2}\ sec^{-1}$ (170 $w\ m^{-2}$, 21 klx) in the 16°C chamber. Fertilizer (28-14-14 Plant Prod Soilless Feed) was applied twice weekly at a rate of 100 mg L^{-1} beginning 2 weeks after planting. Excess fertilizer was leached out with deionized water between fertilizer applications. There were 10 replicates/inoculum treatment/temperature.

Seedlings were harvested when 13 weeks old. Shoot height, root collar diameter, and shoot weights after drying at 80 C, were measured. Nodule number and weight were determined for the whole root system while root length colonized by VAM fungi was estimated from a 10% subsample of the total root weight. Roots were cleared and stained according to Phillips and Hayman (1970) and mycorrhizal infection quantified by the methods of Zak and Parkinson (1982). Roots not used for VAM quantification were dried at 80°C and used to estimate total root weights.

3.3.4 Use of Soil, Nodule and Pure Culture Inocula for Introducing N_2-Fixing Frankia to Containerized Silver-berry

There are numerous methods for introducing Frankia into containerized silver-berry seedlings in the greenhouse; however, the methods vary in their practicality and the efficiency with which the inoculum becomes established. This study was conducted to determine the most effective method for inoculating actinorhizal shrubs on a relatively large-scale basis.

Silver-berry seed was stratified and germinated as discussed previously. The planting mixture consisted of autoclaved peat/vermiculite (50/50, v/v) inoculated as follows:

i) No inoculum - control

ii) Frankia pure culture - Frankia inoculum specific for silver-berry was purchased from Rhizotec Laboratories Inc. in Quebec. Fifty milliliters of the inoculum were diluted in 150 ml deionized water and applied to 15, 7 week old silver-berry seedlings at a rate of 10-15 ml/plant.

iii) Frankia pure culture - Frankia culture SCN 10a was kindly provided by Dr. M. Lalonde, Faculty of Forestry, Laval University, Quebec. The culture was originally isolated from Shepherdia. Frankia was cultured in Qmod media (Quispel, 1960 modified by Carpenter and Robertson, 1983) for 3.5 months and then inoculated with a pipette into the root region of each of 15, 7 week old silver-berry at a rate of 10 ml of Frankia Qmod culture/seedling.

iv) Soil slurry A - soil from the root region of a heavily nodulated silver-berry seedling outplanted on the University of Calgary reclamation plot on the Syncrude site for 1 year was well-mixed and a 25 g subsample placed in 160 ml deionized water. The soil/water slurry was stirred for 2 min and inoculated with a pipette into the root region of 15, 7 week old silver-berry seedlings at a rate of 10 ml/container.

v) Soil slurry B - Twenty-five grams of forest floor soil from beneath buffalo-berry in a mixed poplar-spruce woodland located near the Mildred Lake camp was mixed into 170 ml deionized water and blended for 2 min at 10,000 rpm. The soil slurry was then injected into each seedling as described in iv).

vi) Nodule inoculum A - Fresh nodules were picked from the roots of one year old silver-berry shrubs which had been planted in the University of Calgary reclamation plots (RRTAC pad plot) on the Syncrude site. Approximately 6.4 g wet nodules were washed in deionized water on a 1 mm mesh sieve, crushed to a thick paste in a mortar, and suspended in 150 ml deionized water. Ten milliliters of nodule/water slurry were then inoculated into 7 week old silver-berry seedlings as described in iv).

vii) Nodule inoculum B - Nodules were collected from silverberry shrubs planted by Vaartnou in a reclamation site situated near the Mildred Lake camp. Many of the nodules were found associated with roots permeating rotten wood buried in the sandy soil. Approximately 6.3 g wet weight nodules were

washed, crushed and suspended in deionized water as described in vi). The nodule slurry was inoculated into containerized silver-berry following the method described in iv).

viii) Nodule inoculum C - Fresh nodules were collected from the same source as for vi). Approximately 6 g of wet nodules were rinsed 3 times in deionized water and then mashed in a mortar until they formed a thick paste. The nodule paste was then suspended in polyvinyl pyrrolidine-phosphate buffer solution (a treatment which reduces oxidation of phenols which appear to inhibit the growth of Frankia, Loomis and Battaille, 1966), shaken for 1 min and centrifuged for 10 min at 5000 rpm at 20°C. The supernatent was decanted and the procedure repeated 3 times. The resultant nodule pellets were resuspended in 170 ml deionized water and injected into containerized silver-berry seedlings as described above.

The seedlings were grown in the greenhouse under the conditions detailed in 3.3.2. Two weeks after planting the seedlings began receiving 15-15-18 Plant Prod Soilless Feed fertilizer at a rate of 100 mg L^{-1} twice weekly. Excess fertilizer was flushed out with deionized water between fertilizer applications. The seedlings were grown for 18 weeks after which height, root collar diameter, shoot weight, root weight, nodule number and nodule wet weight were determined for each replicate using the methods described previously.

3.3.5. Effect of Inoculation Method and Inoculation Time on Nodule and Mycorrhizal Development of Buffalo-berry

This study was performed to determine if inoculum soil mixed into the planting mixture was a more effective means of introducing inoculum and promoting symbiont development than applying the inoculum as a soil slurry. Also, the timing of inoculum application was tested by inoculating seedlings of various ages.

Buffalo-berry seed was scarified and germinated as described previously with the exception that seed was treated with sulphuric acid

for 40 rather than 30 minutes. The germinants were planted according to the following treatments:

i) 10 seedlings were planted in sterilized peat/vermiculite

ii) 10 seedlings were planted in peat/vermiculite which had been amended with mixed forest (Mildred Lake camp vicinity) floor soil at a rate of 10% by volume.

iii) 10 seedlings were planted in peat/vermiculite and treated with a mixed forest soil/water slurry. Approximately 18 g of forest soil (same as that used in ii) was mixed with 120 ml deionized water, blended at 10,000 rpm for 2 min and applied to each seedling at a rate of 10 ml/container.

iv) 40 seedlings were planted in peat/vermiculite and 10 seedlings were treated as described above (iii) when they were 2, 3, 4 and 5 weeks old.

Seedlings were grown in a growth chamber programmed for an 18 hour day/6 hour night. Light intensity was measured at 420 $\mu E\ m^{-2}$ (54 klx or 330 $W\ m^{-2}$). After 10 weeks the seedlings were transferred to the greenhouse where daylength was extended to 20 hours and light intensities were in the vicinity of 207 $\mu E\ m^{-2}$ (39.6 klx, 255 $W\ m^{-2}$). Fertilizer was applied at a rate of 200 mg L^{-1} 28-14-14 Plant Prod Soilless Feed once weekly for the first 7 weeks and was increased to 400 mg L^{-1} twice weekly thereafter. The seedlings were harvested after 17 weeks and assessed for shoot height, branching, shoot weight, root weight, nodule weight and mycorrhizal root length as per the methods discussed previously.

3.4 FIELD TRIAL TO TEST GROWTH RESPONSE OF INOCULATED SILVER-BERRY AND BUFFALO-BERRY

In order to determine if the development of an inoculation program is worthwhile, a field trial to assess the growth response of inoculated and uninoculated plants should be conducted. If inoculation confers few benefits on the plant, in terms of growth and survival, inoculation of containerized shrubs prior to outplanting may be unnecessary. Therefore, a study was conducted to determine the effect of inoculating silver-berry and buffalo-berry with soil containing both

VA mycorrhizal and Frankia inoculum on plant growth and symbiont development under field conditions. The seedlings were outplanted on two reconstructed soils, one amended with peat and one amended with peat and clay.

The two University of Calgary plots were located on the east side of the RRTAC soil reconstruction-woody plant experimental area on a specially prepared pad of oil sand tailings from the Syncrude Canada Ltd. extraction plant (Figure 2). The two soil treatments, mixed to a depth of 20 cm, were the application of (1) 11 cm (3% organic C) of muskeg peat (P-1) and (2) 11 cm of muskeg peat plus 2.9 cm (12% clay) of surficial overburden clay (P-2). The plots were 12 x 44 m with buffer strips on all sides leaving 10 x 40 m for planting. The plots were constructed by Hardy Associates in June 1984. The muskeg peat was from the Syncrude NT-2 stockpile and the clay from the J-pit located close to mixed aspen woodland. The clay was from a depth of approximately 1 to 3 m and consisted of 39% clay, 29% silt and 32% sand (Hardy Associates, 1983). Both plots were fertilized with 0-45-0 rock phosphate at a rate of 112 kg ha^{-1} and all amendments thoroughly mixed into the top 20 cm of sand with a Madge Rotoclear machine. Further details on plot construction are given in reports by Hardy Associates.

Each 10 x 40 m plot was staked to delineate 40 rows with 20 planting positions per row. This resulted in 1 m spacing between rows and 0.5 m spacing within rows. Provisions were made to accommodate a total of 10 treatments per plot, each treatment with 4 randomly assigned rows (i.e. 20 plants/row x 4 rows = 80 replicates). The plant species used in the trial were silver-berry, buffalo-berry, green alder and jack pine. Only silver-berry and buffalo-berry will be discussed here.

Silver-berry and buffalo-berry seed were scarified and germinated as outlined previously. The germinants were then planted (65 cc containers) in sterilized peat/vermiculite (50/50, v/v) containing either autoclaved (control treatment) or unautoclaved (inoculated treatment) symbiont inoculum at a rate of 20% by volume. The source of the inoculum was the soil mixture used in the shrub dependency study (3.1.1). The Frankia and mycorrhizal fungi in this soil were

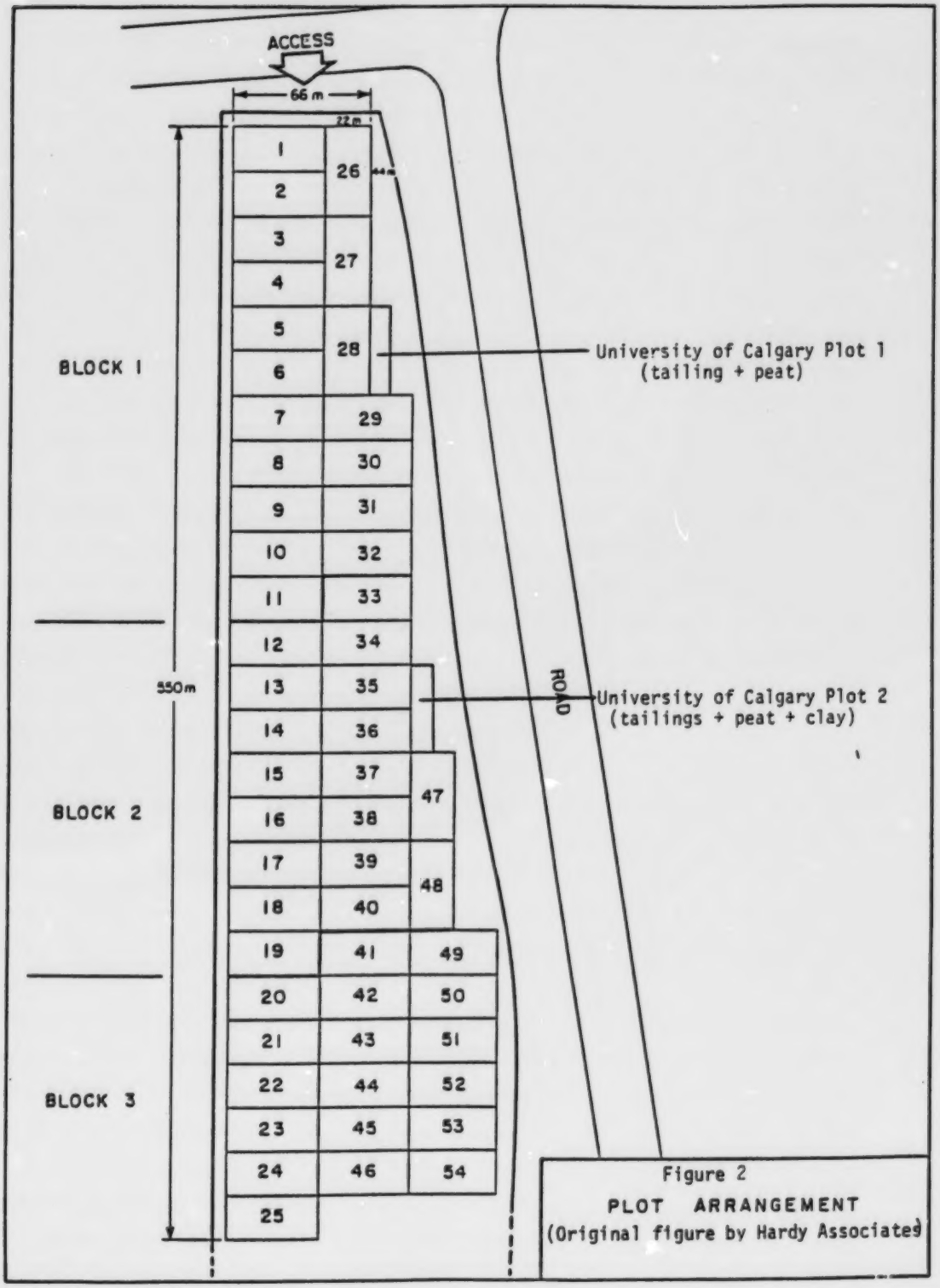

Figure 2
PLOT ARRANGEMENT
(Original figure by Hardy Associates)

propagated in pot culture in the greenhouse by growing silver-berry in a mixture of inoculum soil and peat/vermiculite for 2 months. The pot culture soil was then used to set up the inoculated and uninoculated treatments.

The seedlings were grown in the greenhouse with daylength extended to 20 h and a minimum of 3.5 klx light intensity. They were fertilized at a rate of 100 mg L^{-1} of 15-15-18 Plant Prod fertilizer from weeks 4 to 7 and at a rate of 200 mg L^{-1} during weeks 8 and 9. In week 10 fertilization was reduced to 100 mg L^{-1} and the silver-berry was supplemented with 100 mg L^{-1} of NH_4NO_3. This regime continued until week 13 when buffalo-berry was also supplemented with 100 mg L^{-1} of NH_4NO_3. Both silver-berry and buffalo-berry required additional N to stimulate growth and counteract chlorosis. In weeks 15 and 16, fertilizers were applied only once per week. Fertilization was stopped in week 17 and the seedlings were hardened off outdoors for two weeks prior to outplanting.

Preplanting mycorrhizal and nodulation assessments were made by randomly selecting 10 plants from each species-inoculation treatment, removing the shoots, washing the planting mixture from the roots and determining shoot weight, root weight, VA mycorrhizal status and nodule status. The methods used have been presented previously.

Survival was measured in the field one year after outplanting. In each of the inoculated and uninoculated treatments in each of the two plots, 10 randomly chosen seedlings/species were excavated and transported to the laboratory. Excavation consisted of digging an approximately 25 cm square to a depth of 20 cm around each plant, shaking the excess sand from the roots and placing the plant in a plastic bag. The shoots were clipped at the root/shoot interface and shoot height and weight (after drying at 80°C) were measured. The root systems were washed free of soil, nodules were counted, separated from the roots and weighed. Five of the 10 root systems in each treatment were selected randomly and subsampled for VA mycorrhizal assessments. The size of the subsamples varied with the size of the root system and ranged from 15% of the total wet weight for large root systems to 50% of the total for small root systems. Only relatively young roots (2 mm diameter or less) were sampled since older, thicker

roots usually lacked a cortex - the site of VA mycorrhizal infection. The roots were cleared (8 min) and stained (7 min) following the methods of Phillips and Hayman (1970) and mycorrhizal infection quantified as outlined by Zak and Parkinson (1982). The remaining roots were dried at 80°C and dry weights determined.

Silver-berry foliage was analyzed for total N and P. All 10 replicates for each inoculated and uninoculated treatment in each plot were ground in a Wiley rotary mill to pass a 40 mesh (425 μm) screen. The samples were digested with concentrated sulphuric acid and 30% hydrogen peroxide in a Technicon BD-block digester. Acid digests were filtered through a Whatman No. 1 filter and stored. Both total N (as ammonium N) and P (as orthophosphate P) were determined colorimetrically on a Technicon Autoanalyzer II system using the ammonium molybdate/ascorbic acid chemistry for PO_4-P and the Berthelot Reaction for NH_4-N.

Sampling was repeated two years after outplanting. Due to poor survival (particularly in the inoculated treatment) during the first winter after outplanting, the number of surviving plants in each treatment in each plot was often less than 10. To increase replication, shrubs from both plots were pooled resulting in 15 replicate silver-berry and 7 replicate buffalo-berry plants in each of the inoculated and uninoculated treatments. It was felt that pooling the silver-berry from both plots was justified since plot treatment effects on shrub growth and nodule development were insignificant for this species after the first year. Although buffalo-berry exhibited plot treatment effects on growth and nodule development after the first growing season, pooling of second year plants was necessary to improve replication so statistical analysis could be performed.

Two-year old plants were excavated and shoot heights, shoot weights, root weights, nodule weights and mycorrhizal colonization assessed as described for the one-year old plants. In addition, root collar diameters and frequency of branching was measured at this sample time.

4. RESULTS

4.1 MYCORRHIZAL STATUS OF WOODY SHRUBS

Buffalo-berry, silver-berry, saskatoon-berry and cinquefoil were all found to be strictly vesicular-arbuscular mycorrhizal (VAM) regardless of sampling location (Table 1). Mycorrhizal infection of young, active roots (dia. $\leq$2 mm) ranged up to 60% for both buffalo-berry and silver-berry and was slightly higher for saskatoon-berry and lower for cinquefoil.

4.2 JUSTIFICATION FOR INOCULATION OF CONTAINERIZED SILVER-BERRY AND BUFFALO-BERRY

4.2.1 The Dependency of Silver-berry and Buffalo-berry on their Mycorrhizal and N_2-Fixing Symbionts

Shoot weights were 4 and 9 times greater for inoculated silver-berry and buffalo-berry, respectively, than for their uninoculated counterparts, while the roots of the inoculated shrubs weighed 3 and 4 times more, respectively, than the roots of the uninoculated shrubs (Table 2). In the inoculated treatments, nodule development was more pronounced on the buffalo-berry than the silver-berry, but VAM colonization was approximately the same for the two shrub species (i.e. 64 and 70%). Low levels of nodulation and mycorrhization were detected in the uninoculated treatments suggesting contamination of the planting mixture occurred during the course of the experiment.

4.2.2 Levels of VA Mycorrhizal Inoculum in Various Soils in the Fort McMurray, Alberta Region

Although slender wheatgrass was used to assay various soils for VAM inoculum potential, the results should be applicable to buffalo-berry and silver-berry since both the grass and the shrubs are VA mycorrhizal and evidence to date suggests that VAM fungi are generally not host specific. Because grasses grow faster and, due to the fibrosity of their roots, tend to exploit a greater volume of soil than many shrub species do, they offer a more rapid and efficient means for surveying soils for VAM inoculum.

Table 1. Mycorrhizal status of selected woody shrubs growing in the Fort McMurray and Kananaskis, Alberta regions. VAM = vesicular-arbuscular mycorrhizae.

Shrub species	Sampling Location and Date	Adjacent Woodland[1]	Mycorrhizal Status	Infection density (%)
Buffalo-berry	Vaartnou plot, Mildred L. camp (June, n = 5)	Jack pine/ lichen	VAM	Most roots dead Infection low, 5-10%
Buffalo-berry	Cutbank near Suncor plant (Oct., n = 5)	Mixed/ aspen	VAM	20-40
Buffalo-berry	Kananaskis, roadcut (Aug., n = 5)	Mixed/ aspen	VAM	20-60
Silver-berry	Vaartnou plot, Mildred L. camp (June, n = 5)	Jack pine/ lichen	VAM	20-50
Silver-berry	Kananaskis (Aug., n = 5)	Mixed/ aspen	VAM	40-60
Saskatoon-berry	Cutline near Mildred L. camp (Oct., n = 5)	Jack pine/ lichen	VAM	60-80
Cinquefoil	Vaartnou plot (June, n = 5)	Jack pine/ lichen	VAM	20-30

[1] Woodland located in close vicinity to sampling location.

Table 2. Shoot and root production and symbiont development in silver-berry and buffalo-berry grown in reconstructed soils with and without symbiont inoculum. Data are means ± SD.[1]

Shrub	Inoculum	Shoot Weight (mg dwt)	Root Weight (mg dwt)	Nodule Weight (mg wet wt)	VAM Colonization (%)
Silver-berry	+	422 ± 21[b]	139 ± 23[b]	6 ± 3[b]	70 ± 16[b]
	-	108 ± 27[a]	45 ± 12[a]	1 ± 1[a]	1 ± 2[a]
Buffalo-berry	+	244 ± 51[b]	72 ± 25[b]	44 ± 20	64 ± 6[b]
	-	27 ± 9[a]	17 ± 8[a]	0	2 ± 3[a]

[1] Data analyzed by a two sample T-test. Values in each column followed by the same letter for either silver-berry or buffalo-berry are not significantly different (p = 0.05).

Vesicular arbuscular mycorrhizal inoculum potential was negligible (0-5% VAM infection) in the majority of peat samples (Table 3). Peat, stockpiled for 8 to 12 months on the Syncrude site, lacked VAM inoculum, but increased levels of inoculum were evident in stockpiled peat which had been revegetated with a grass/legume (VAM hosts) mixture for 6 years. Mixed woodland soil exhibited the highest VAM inoculum potential with coarse textured soil from an aspen/shrub/grass woodland being the most infective (64% mycorrhizal infection) of all the soils tested.

4.2.3 Growth Characteristics and Mycorrhizal Potential of Undisturbed Bog Peat and Stockpiled Peat

Again, slender wheatgrass, rather than a shrub species, was used in this VAM assay.

Table 3. Vesicular-arbuscular mycorrhizal (VAM) infection of slender wheatgrass grown in the greenhouse in various soils collected from the Fort McMurray, Alberta region. Infection expressed as % total root length infected.

Soil Description	Age of Plant When Sampled (wk)	% VAM Infection
Aspen woodland mineral	8	13.0
Fine textured soil from beneath undisturbed mixed woodland	12	37.0
Coarse textured soil from beneath undisturbed mixed woodland	12	64.0
Carex/Sphagnum peat mixture from Syncrude and Suncor leases	9	1.6
Undisturbed peat, Canstar lease	12	0.6
Undisturbed peat, 0-15 cm depth, Syncrude lease	12	14.5
Undisturbed peat, 50-100 cm depth, Syncrude lease	12	4.8
NT 2 (Syncrude) stockpile peat stockpiled for:		
8 mo., 0- 15 cm depth	12	0
8 mo., 50-100 cm depth	12	3.1
12 mo., 0- 15 cm depth	8	0.2
Peat stockpiled for 6 years on Syncrude site (East Muskeg)	9	13.7

Shoot weights, root weights, shoot/root (S/R) ratios, total root lengths and % mycorrhizal infection were very similar in the undisturbed and stockpiled peat (Table 4). There was very little effect of sampling depth on the majority of parameters tested, with the exception of shoot weights, which were greater in the 0 - 15 cm deep undisturbed and stockpiled peat than in the 50 - 100 cm deep peat. Percent VAM colonization was highest in the undisturbed surface peat, but not significantly so since sample variation was high (Appendix Table 1). Mycorrhizal inoculum levels were generally very low with the infection consisting mainly of hyphae and arbuscules (Appendix Table 1).

4.2.4 Mycorrhizal Potential of Revegetated Dyke Peat

Slender wheatgrass shoot and root weights and root lengths were slightly lower in 6 year-old stockpiled peat than 8 month-old stockpiled peat, while the reverse was true for VA mycorrhizal inoculum potential (Tables 4, 5). Fertilization of the 6 year-old stockpiled peat significantly improved plant productivity while not significantly altering VAM inoculum potential (Table 5).

4.2.5 Effect of VA Mycorrhizal Inoculation on Plant Performance of Slender Wheatgrass Grown in Stockpiled Peat Under Fertilized and Unfertilized Conditions

Inoculation of slender wheatgrass with the VAM fungus, Glomus aggregatum, significantly improved shoot and root production but only if the plants received no fertilizer and only when the plants were 10 weeks old (Table 6). As expected, fertilization greatly improved plant growth but counteracted the potentially beneficial effects of the VAM fungus, resulting in very few differences between inoculated and uninoculated treatments at the two sample times.

4.2.6 Mycorrhizal and Nodule Status of Containerized Shrubs Planted on the Oil Sands Tailings Reconstruction Plots

Shoot and root weights of the containerized VAM shrub species planted on the RRTAC oil sands tailings reconstruction plots (i.e. pad plots) are given in Table 7. Buffalo-berry and silver-berry shoots appeared to be underweight and the symbiont status of all the shrubs

Table 4. Characteristics of slender wheatgrass grown in peat from a muskeg bog and peat stockpiled for eight months.[1]

Plant Parameter	Peat Source	Sampling Depth (cm) 0 - 15	50 - 100
Shoot weight (mg dwt plant^{-1})	Undisturbed	94.3^{b}	54.4^{a}
	Stockpile	73.9^{b}	59.7^{a}
Root weight (mg dwt plant^{-1})	Undisturbed	127.9^{b}	96.2^{a}
	Stockpile	97.2^{a}	100.1^{a}
Shoot/root ratio	Undisturbed	0.74 (.23)	0.57 (.09)
	Stockpile	0.77 (.15)	0.60 (.13)
Total root length (m L^{-1})	Undisturbed	545.0^{a}	578.6^{a}
	Stockpile	563.5^{a}	623.1^{a}
Percent VAM infection	Undisturbed	14.5^{a}	4.8^{a}
	Stockpile	0^{a}	3.1^{a}

[1] Shoot and root weight data analyzed by a Kruskal-Wallis test. Root length and VAM infection analyzed by two-way ANOVA. Values in each data set followed by the same letter are not significantly different ($p = 0.05$). Values in brackets are standard deviations.

Table 5. Root and shoot production by slender wheatgrass grown in the greenhouse in fertilized and unfertilized dyke peat. Peat had been stockpiled for 6 years prior to spreading on the dyke.[1]

Measurement	Treatment	
	Unfertilized	Fertilized
Shoot weight (mg plant^{-1})	55[a]	144[b]
Root weight (mg plant^{-1})	81[a]	139[b]
Shoot/Root	0.68	1.04
Root length (m L^{-1} soil)	326[a]	508[b]
Mycorrhizae (%)	14[a]	16[a]

[1] Data analyzed by Hotelling's T^2 test. Means in each row not followed by the same letter differ significantly ($p = 0.05$).

was poor. With the exception of one pincherry plant, which exhibited a small patch of mycorrhizal infection caused by the "fine endophyte", all the shrubs were nonmycorrhizal. None of buffalo-berry and 16% of the silver-berry were nodulated. The fungal root pathogen, Thielaviopsis, was occassionally observed in the silver-berry roots while Olpidium, another fungal parasite, occurred frequently in the roots of all the shrub species.

Table 6. Shoot and root production by slender wheatgrass grown in stockpiled peat (50-100 cm deep) inoculated with Glomus aggregatum and fertilized or left unfertilized.[1]

Measurement		4 week old plants		10 week old plants	
		Fertilized	Unfertilized	Fertilized	Unfertilized
Shoot weight	Inoculated	22^{c}	8^{ab}	132^{d}	22^{c}
(mg dwt plant^{-1})	Uninoculated	14^{bc}	7^{a}	113^{d}	12^{ab}
Root weight	Inoculated	22^{b}	10^{a}	194^{d}	30^{b}
(mg dwt plant^{-1})	Uninoculated	18^{b}	10^{a}	146^{c}	11^{a}

[1] Data for each parameter analyzed by three-way ANOVA (MSE = .0715 and .1077 for shoots and roots respectively). A three-way interaction was observed for shoot data, hence Scheffé multiple contrasts were applied to individual treatment means. No three-way interaction was observed for root data, hence Scheffé multiple contrasts were applied to two-way means. Values within each data set followed by the same letter(s) do not differ significantly (p = 0.05). Data required LN (Y + 1) transformation.

4.2.7 Growth Characteristics and Symbiont Status of Woody Shrubs Raised in Various Commercial Nurseries in Alberta and British Columbia

Shoot heights, shoot weights, root weights and S/R ratios for all species surveyed are summarized in Table 8. Shoot weights for each plant species varied greatly amongst nurseries mainly due to differences in crop year, growing regimes (i.e. fertilization rates, light conditions) and possibly container size. Silver-berry from the Laidlaw Nursery were of an acceptable size (0.98 g) while those from

Table 7. Mycorrhizal and nodule status of shrub species planted on the RRTAC oil sand tailings reconstruction plots in September, 1984. Data are means (n=25) ± SD.

Species	Grower	Date started	Plant wt (g dwt) Shoot	Root	Total	Mycorrhizal Status[1]	Nodulated plants/25
Rosa sp. Wild rose	L[2]	April 1984	0.70 ± 0.34	1.31 ± 0.57	2.01 ± 0.80	-	NA[2]
Prunus pensylvanica L.F. Pin cherry	L	April 1984	1.06 ± 0.54	1.47 ± 0.75	2.52 ± 1.17	±	NA
Amelanchier alnifolia Nutt. Saskatoon	S[2]	January 1983	3.40 ± 1.00	1.79 ± 0.66	5.19 ± 1.42	-	NA
Shepherdia canadensis (L.) Nutt. Canada Buffalo-berry	L	April 1984	0.53 ± 0.21	0.25 ± 0.08	0.77 ± 0.29	-	0
Elaeagnus commutata Bernh. Silver-berry	L	April 1984	0.46 ± 0.20	0.22 ± 0.10	0.68 ± 0.29	-	4

[1] One small patch of VAM infection was observed in the root system of one pincherry plant. The mycorrhizal fungus was identified as the "fine endophyte." In general, roots were in good condition, although extensive infection by Thielaviopsis, a root pathogen was observed in two of the silver-berry plants. Olpidium, another root parasite, was observed regularly in the roots of all species.

[2] NA = not applicable to this host; L = Laidlaw; S = Syncrude.

Table 8 Size of container-grown and bareoot woody shrubs obtained from four commerical nurseries in August, 1985. Data are means ± SD.

Plant species	Nursery	Crop	Container size (cc)	No. of seedlings	Shoot Height (cm)	Shoot Weight (g)	Root Weight (g)	S/R (range)
Pincherry	Reid-Collins	Unknown	SL 47(?)	8	NM	1.58 ± 0.43	2.26 ± 0.63	.7 (.5 - 1.5)
	Whitecourt	Heeled in ('84)	NA	2	60, 46	17.64, 8.16	NA	NA
	Laidlaw	1984	SL 150	10	38 ± 8	1.64 ± 0.5	1.76 ± 0.48	.9 (0.6 - 1.4)
	Laidlaw	1985	SL 150	10	46 ± 10	1.91 ± 0.85	1.36 ± 0.55	1.4 (0.9 - 1.8)
Saskatoon	Reid Collins	Unknown	SL 47(?)	10	NM	1.70 ± 0.56	1.97 ± 0.95	0.9 (0.6 - 1.5)
	Whitecourt	1984	Styro-20	10	35 ± 4	4.46 ± 1.19	2.01 ± 0.58	2.3 (2.0 - 3.4)
	Whitecourt	1985	Styro-4	10	21 ± 4	1.84 ± 0.53	NM	NM
	Laidlaw	1985	SL 150	10	17 ± 4	1.42 ± 0.41	0.83 ± 0.37	2.0 (1.0 - 3.7)
	Oliver	1983	SL 150	10	30 ± 11	2.36 ± 1.41	1.48 ± 0.92	1.7 (1.0 - 2.3)
	Oliver	3 - 5 y(?)	Bareroot	10	35 ± 5	NA[1]	NA[1]	NA[1]
Dogwood	Oliver	1985	SL 150	10	18 ± 3	0.46 ± 0.13	0.18 ± 0.06	2.5 (2.0 - 3.6)
Willow	Reid-Collins	1985(?)	SL 150	10	NM	NA[2]	0.18 ± 0.08	NM
Cinquefoil	Whitecourt	1984	Styro-8	10	39 ± 7	2.62 ± 1.61	0.68 ± 0.56	4.6 (2.5 - 7.6)
Silver-berry	Reid-Collins	?	SL 47(?)	10	NM	0.39 ± 0.06	0.18 ± 0.07	2.8 (1.1 - 3.3)
	Laidlaw	1985	SL 150	10	23 ± 6	0.98 ± 0.48	0.39 ± 0.24	2.8 (1.3 - 6.1)
	Oliver	1983	SL 150	10	19 ± 3	0.77 ± 0.29	0.30 ± 0.12	2.7 (1.8 - 3.2)
Buffalo-berry	Reid Collins	?	SL 47(?)	10	NM	1.11 ± 0.53	1.97 ± 0.77	0.6 (0.4 - 0.9)
	Syncrude[3]	1981	?	10	NM	0.59 ± 0.16	0.58 ± 0.19	1.0
		1982	?	10	NM	0.57 ± 0.23	0.57 ± 0.17	1.0
		1983	?	10	NM	0.11 ± 0.04	0.12 ± 0.03	0.9
Silver buffalo-berry	Oliver	1985	SL-150	10	12 ± 1	0.18 ± 0.04	0.05 ± 0.02	3.7 (2.6 - 6.3)
	Oliver	3 - 5 yr(?)	Bareroot	10	36 ± 4	NA[1]	NA[1]	NA[1]
	Syncrude[4]	1983	?	10	NM	0.09 ± 0.04	0.07 ± 0.03	1.3
Russian olive	Oliver	3 - 5 yr (?)	Bareroot	10	32 ± 4.3	NA[1]	NA[1]	NA[1]

[1] Seedlings pruned

[2] Cutting included in shoot weight determination

[3] 1981 crop sampled in June 1984; 1982 and 1983 crops sampled in October, 1983

[4] Sampled in June 1984

NM = not measured; NA = not applicable; SL = Spencer Lemaire; Styro = Styroblock

the Reid-Collins Nursery were unusually small (0.39 g) in comparison. The 1981 and 1982 buffalo-berry from the Syncrude Nursery also tended to be underweight and silver buffalo-berry from the same nursery were particularly stunted. The shoot weights of the actinorhizal shrubs were generally less than those of the non-actinorhizal shrubs of equivalant age. Shoot/root ratios were highly variable, ranging from 0.6 (Reid-Collins buffalo-berry) to 4.6 (Whitecourt cinquefoil).

Seedlings sampled in the same year that they were planted were usually non-mycorrhizal (Table 9). Seedlings which were one year old or more and had probably overwintered in the shadehouse or outdoors were quite often mycorrhizal (Reid-Collins pincherry, saskatoon, buffalo-berry, Whitecourt saskatoon, Oliver saskatoon) but not always so (Laidlaw pincherry, Oliver silver-berry, Syncrude buffalo-berry). The bareroot stock was heavily mycorrhizal. The "fine endophyte", characterized by its narrow hyphae (2-3 µm dia) and finely branched arbuscules, was frequently observed on the roots of saskatoon (Whitecourt) and pincherry (Reid-Collins, Laidlaw '85 pincherry). Many of the seedlings were infected with the fungal root parasite, Olpidium, regardless of nursery or seedling age. Thielaviopsis, a pathogenic fungus which causes black root rot of tobacco and many vegetables, occurred in 60% of the silver-berry from the Laidlaw nursery although this was not evident from the condition of the shoot.

There was no evidence of nodulation on the actinorhizal shrubs unless the shrubs were older than one year or were bareroot stock.

4.2.8 Rates of Mycorrhization and Nodulation in Buffalo-berry Grown in Woodland Soil and Amended Tailings Sand in the Greenhouse

Shoot and root weights increased with time in both soil treatments and, by the end of the study, were significantly greater in the woodland soil than in the tailings sand (Table 10). Seedlings raised in woodland soil became mycorrhizal much more rapidly (25% infection at 2 weeks) than seedlings grown in amended tailings sand (no infection until 8 weeks after planting). Percent mycorrhizal infection increased significantly with time in the woodland soil but not in the tailings sand where it remained relatively stable. A significantly greater amount of VAM infection was attained in the woodland soil (87%) than in

Table 9. Vesicular-arbuscular mycorrhizal (VAM) status and *Frankia* nodule development in woody shrubs samples from four commercial nurseries in 1985.

Plant species	Nursery	Crop	No. of Seedlings Surveyed	% Seedlings Mycorrhizal	% VAM[1] (range)	% Seedlings Nodulated	Nodule No. Plant^{-1}	Nodule Weight (g wet plant^{-1})
Pincherry	Reid-Collins	Unknown	8	63	<1 - 20	NA	NA	NA
	Whitecourt	Heeled in (1984)	2	100	33	NA	NA	NA
	Laidlaw	1984	8	0	0	NA	NA	NA
	Laidlaw	1985	10	30	<1 - 2	NA	NA	NA
Saskatoon	Reid-Collins	Unknown	10	50	<2 - 73	NA	NA	NA
	Whitecourt	1984	10	100	<1 - 58	NA	NA	NA
	Laidlaw	1985	10	0	0	NA	NA	NA
	Oliver	1983	10	40	<1 - 66	NA	NA	NA
	Oliver	3-5 y (?)	10	100	40 - 60	NA	NA	NA
Dogwood	Oliver	1985	10	0	0	NA	NA	NA
Willow	Reid-Collins	1985 (?)	10	0	0	NA	NA	NA
Cinquefoil	Whitecourt	1984	10	0	0	NA	NA	NA
Silver-berry	Reid-Collins	Unknown	25	0	0	0	0	0
	Laidlaw	1985	10	0	0	0	0	0
	Oliver	1983	10	0	0	10	4	NM
Buffalo-berry	Reid-Collins	Unknown	25	20	<1 - 33	88	NM	0.27 ± 0.24
	Syncrude[2]	1981	10	0	0	60	1 - 3	0.019 - 0.098
		1982	10	0	0	20	NM	NM
		1983	10	0	0	0	0	0
Silver Buffalo-berry	Oliver	1985	10	0	0	0	0	0
		3 - 5 y (?)	Bareroot	100	50 - 80	100	22 ± 13	0.47 ± 0.26
	Syncrude[3]	1983	5	0	0	0	0	0
Russian olive	Oliver	3 - 5 y (?)	Bareroot	100	40 - 70	100	6 ± 3	0.12 ± 0.10

[1] % VAM refers to % of fine (<5 mm diameter) roots infected with VAM
[2] 1981 crop sampled in June, 1984; 1982 and 1983 crops sampled in October, 1983
[3] sampled in June 1984
NA = not applicable; NM = not measured

Table 10. Rates of shoot and root production, mycorrhizal colonization and nodulation by buffalo-berry grown in undisturbed woodland soil and peat/clay amended tailings sand. Data are means (n = 5) ± SD[1].

Age (wk)	Shoot wt (mg)		Root wt (mg)		% Mycorrhizal colonization		Nodule wt (mg wet)	
	Woodland	Tailings	Woodland	Tailings	Woodland	Tailings	Woodland	Tailings
2	17 ± 3^{a}	14 ± 2^{a}	2 ± 1^{a}	1 ± 1^{a}	25 ± 31^{ab}	0	0	0
4	20 ± 4^{a}	19 ± 1^{a}	2 ± 1^{a}	2 ± 1^{ab}	18 ± 28^{a}	0	0	0
8	52 ± 10^{ab}	48 ± 11^{b}	5 ± 2^{a}	5 ± 2^{b}	57 ± 22^{ab}	23 ± 35^{a}	+	+
12	130 ± 82^{b}	65 ± 30^{b}	25 ± 18^{b}	8 ± 2^{c}	67 ± 27^{ab}	33 ± 21^{a}	64 ± 12^{a}	6 ± 8^{a}
20	367 ± 156^{c}	172 ± 96^{c}	308 ± 148^{c}	114 ± 95^{d}	87 ± 10^{b}	26 ± 6^{a}	85 ± 7^{b}	34 ± 14^{b}

[1] Data in each column analyzed by one-way ANOVA. Root and shoot weight data required LN transformations. Values in each column followed by the same letter(s) are not significantly different ($p \leq 0.05$).

the tailings sand (33%). The mycorrhizal data were highly variable suggesting a high degree of variation in VAM inoculum potential amongst the replicate soil samples. Nodules were first visible at 8 weeks when 100% of the woodland soil buffalo-berry had formed nodules compared with 40% for seedlings planted in the tailings sand. Seedlings, older than 8 weeks, were all nodulated with nodules produced on woodland seedlings weighing more than those produced on tailings seedlings. Nodulation increased with seedling age.

4.2.9 Rates of Mycorrhizal and Nodule Development in Silver-berry Outplanted in an Undisturbed Woodland and the Suncor Tar Island Dyke

Shoot weights did not increase over the growing season in any of the planting locations; in fact, there tended to be a loss in weight between weeks 6 and 12 due to leaf abscission (Table 11). Root growth was also negligible, as was evident from the lack of root growth out of the planting plug in all treatments except the dyke plot revegetated in 1978. Seedling survival over the first 6 weeks after outplanting was higher in the undisturbed mixed woodland (100%) than in the dyke plots, particularly in the plot revegetated in 1974 where only 36% of the seedlings were alive after 6 weeks. Vegetation in the 1974 plot was dominated by sweet clover.

Nodulation was minimal after 6 weeks although 2 plants in the 1978 dyke plot possessed small nodules. After 12 weeks all the seedlings in the undisturbed woodland plot had become nodulated, but, with the exception of the 1978 dyke plot where 4 plants became nodulated, none of the seedlings planted on the dyke formed nodules. Nodule number was highest on plants from the woodland plot. Almost all seedlings, regardless of planting location, became mycorrhizal within 6 weeks of being outplanted. Percent mycorrhizal infection, however, was patchy, possibly a result of poor root growth out of the planting plug.

Table 11. Growth, nodulation and VA-mycorrhizal development in uninoculated silver-berry out-planted in the boreal forest and Suncor Tar Island Dyke for 6 and 12 weeks. Dyke locations revegetated in 1971, 1974, 1978. Data are means ± SD[1].

Measurement	Age (wk)	Preplant	Planting locations			
			Undisturbed	Dyke 1971	Dyke 1974	Dyke 1978
Shoot weight	6	216 ± 68^{a}	229 ± 84^{a}	195 ± 58^{a}	205 ± 50^{a}	230 ± 80^{a}
(mg dry plant^{-1})	12	216 ± 68^{b}	153 ± 34^{ab}	111 ± 42^{a}	158 ± 47^{ab}	176 ± 63^{ab}
Root weight	6	119 ± 34^{b}	80 ± 27^{ab}	87 ± 24^{ab}	73 ± 24^{a}	94 ± 37^{a}
(mg dry plant^{-1})	12	119 ± 34^{a}	145 ± 23^{a}	83 ± 34^{a}	119 ± 66^{a}	127 ± 67^{a}
Survival/50	6	NA	50	38	18	43
Root growth	6	NA	0	0	0	0
out of plug	12		0	0	0	4/10
Plants with	6	0	0/10	0/10	0/10	2/10
nodules	12	0	10/10	0/10	0/ 7	4/10
Nodules/plant	6	0	0	0	0	1
(wet wt plant^{-1})	12	0	4-21 (.006-.028)	0	0	1-8
Plants with	6	0	10/10	9/10	10/10	8/10
VAM	12	0	10/10	9/10	7/ 7	10/10
% VAM	6	0	<1-58	<1-71	5-31	5-29
	12	0	<1-20	5-70	<2-30	1-20

[1] Shoot and root weight data analyzed by one-way ANOVA and differences detected by Scheffé multiple contrasts for pairwise comparisons. Values in each row followed by the same letter(s) are not significantly different ($p = 0.05$).

4.3 DEVELOPMENT OF A GROWING REGIME FOR GREENHOUSE PRODUCTION OF MYCORRHIZAL, NODULATED SILVER-BERRY AND BUFFALO-BERRY

4.3.1 Fertilizer Effects on Growth, Nodulation and Mycorrhizal Development in Buffalo-berry and Silver-berry

Shoot production by buffalo-berry was greater in the woodland soil than in the tailings sand but only in the 0 and 100 mg L^{-1} fertilizer regimes (Table 12). At the higher fertilizer regimes (200 and 400 mg L^{-1}) shoot production in the two soils was similar. There was an increasing trend in shoot weights with increasing fertilization for plants in the tailings sand but not for plants in the woodland soil where no significant differences in shoot weights were detected amongst the three fertilizer rates tested. Shoot response of silver-berry was similar to that of buffalo-berry (Table 13), with the exception that the 400 mg L^{-1} fertilizer regime greatly stimulated shoot production, particularly in the tailings sand treatment.

The patterns of root production at the various fertilizer regimes were very similar for the two shrub species (Tables 12, 13). Overall, root weights were lower in the tailings sand than in the woodland soil. Fertilization at 200 mg L^{-1} significantly increased buffalo-berry root production, while silver-berry root weights were 1.7 and 2.0 times greater at the 400 mg L^{-1} rate than at the 0 and 100 mg L^{-1} rates, respectively. Silver-berry plants were generally heavier than buffalo-berry plants.

At the 0 and 100 mg L^{-1} fertilizer regimes, almost all the buffalo-berry and silver-berry seedlings in both test soils developed nodules. At 200 mg L^{-1} fertilizer (56 mg N), nodule formation based on nodule weight, appeared to be inhibited but not significantly so. However, application of 400 mg L^{-1} fertilizer or 112 mg N significantly reduced nodule production rate on both species in both soil types. Although nodule formation was reduced at the high fertilizer regime, all the silver-berry and the majority of buffalo-berry in the woodland soil became nodulated.

Mycorrhization appeared to be less sensitive to fertilization than nodulation. The majority of seedlings at all fertilizer treatments in both soils developed mycorrhizae, but the extent of

Table 12. Fertilizer effects on growth, nodulation, and mycorrhizal development in buffalo-berry grown in woodland soil and peat/clay-amended tailings sand. Age = 20 weeks.[1]

Measurement	Soil	Fertilizer (mg/L 28-14-14)				Row Means
		0	100	200	400	
Shoot weight (mg)	Woodland	377^{bc}	473^{bcd}	497^{cd}	552^{cd}	NA
	Tailings	144^{a}	269^{ab}	519^{cd}	664^{d}	NA
Root weight (mg)	Woodland	239	234	404	388	316^{b}
	Tailings	51	178	273	288	198^{a}
	Column means	145^{a}	206^{a}	339^{b}	338^{b}	
% Seedlings with nodules	Woodland	100	100	80	100	
	Tailings	100	80	20	40	
Nodule weight (mg wet)	Woodland	60	34	11	7	28^{b}
	Tailings	24	22	6	3	14^{a}
	Column means	42^{b}	28^{b}	8.5^{ab}	5^{a}	
% Seedlings with mycorrhizae	Woodland	100	100	100	100	
	Tailings	100	80	100	80	
% Mycorrhizal infection	Woodland	94	90	82	40	77^{b}
	Tailings	46	56	73	23	50^{a}
	Column means	70^{b}	73^{b}	78^{b}	32^{a}	

[1] Data analyzed by two-way ANOVA. Differences amongst means were detected by Scheffé multiple contrasts for pairwise comparisons applied to individual means where a significant interaction occurred or to row or column means if no interaction was detected in the ANOVA. Values in each data set followed by the same letter are not significantly different. (MSE for shoot weight, root weight, nodule weight, and % mycorrhizal infection are 12040, 7944.9, 270.21, and 515.28, respectively.)

Table 13. Fertilizer effects on growth, nodulation, and mycorrhizal development in silver-berry grown in woodland soil and peat/clay-amended tailings sand. Age = 20 weeks.[1]

Measurement	Soil	Fertilizer (mg/L 28-14-14)				Row Means
		0	100	200	400	
Shoot weight (mg)	Woodland	515	637	586	622	590[b]
	Tailings	286	399	453	863	500[a]
	Column means	401[a]	518[a]	520[a]	743[b]	
Root weight (mg)	Woodland	337	334	444	507	406[b]
	Tailings	257	174	230	523	296[a]
	Column means	297[a]	254[a]	337[ab]	515[b]	
% Seedlings with nodules	Woodland	100	100	100	100	
	Tailings	100	100	50	40	
Nodule weight (mg wet plant^{-1})	Woodland	91	58	30	15	49[a]
	Tailings	42	76	64	12	49[a]
	Column means	67[b]	67[b]	47[ab]	14[a]	
% Seedlings with mycorrhizae	Woodland	100	100	100	100	
	Tailings	100	100	50	80	
% Mycorrhizal infection	Woodland	94	90	93	85	91[a]
	Tailings	37	52	29	17	34[b]
	Column means	66[a]	71[a]	61[a]	51[a]	

[1] Data analyzed by two-way ANOVA. Shoot weight data required a LO transformation (MSE = .02788) while root weight data required a SQRT transformation (MSE = 11.319). MSE for nodule weight and mycorrhizal infection = 1273.9 and 298.71, respectively. No significant interaction was detected, hence Scheffé multiple contrasts for pairwise comparisons were applied to row and column means. Values in either the row or column means for each measurement followed by the same letter are not significantly different (p = 0.05).

mycorrhizal colonization was significantly less in the tailings sand than in the woodland soil. Percent mycorrhizal infection in the buffalo-berry was significantly reduced at the high fertilizer rate, but mycorrhizal formation in the silver-berry was not inhibited at any of the fertilizer rates.

Based on these data, it appears that the fertilizer concentration required to produce a mycorrhizal, nodulated silver-berry or buffalo-berry should not exceed 56 mg N L^{-1}.

4.3.2 Effect of Container Volume and Inoculation on Growth of Silver-berry

Both 12 and 20 week-old seedlings were significantly larger and heavier when grown in 150 cc containers than when grown in 65 cc containers (Table 14). Shoot and root weights of inoculated plants were significantly greater than those of the uninoculated plants, but this was the case only when plants were grown in the 150 cc containers. Plants grown in the 65 cc containers demonstrated no significant response to inoculation.

Almost all the seedlings became nodulated, including those in the uninoculated treatments, suggesting that there was some cross-contamination of the *Frankia* symbiont between treatments. All the plants in the inoculated treatments became mycorrhizal, but percent infection was low (20-25%) and variable. None of the uninoculated plants became mycorrhizal and container size did not seem to influence mycorrhizal development.

4.3.3 Growth of Silver-berry as Influenced by Soil Temperature and Symbiont Inoculation

Shoot heights, shoot weights and root weights were, with the exception of roots in the *Glomus aggregatum* treatment, significantly greater at 26°C than at 16°C (Table 15). Root collar diameters were also greater at 26°C but only for seedlings inoculated with woodland and silver-berry soil. The largest, heaviest plants with the heaviest root systems were obtained in the 26°C, woodland soil-inoculated treatment (shoot ht = 24 cm, shoot wt = 966 mg, root wt = 424 mg). Inoculation with silver-berry soil also stimulated shoot production but not to the same

Table 14. Effect of container volume and inoculation on shoot and root production by silver-berry. Plants received 200 mg L^{-1} 15-15-18 fertilizer twice weekly.

Plant age (weeks)	Container Volume (cc)	Inoculum	Plant Weight (mg dwt) Shoot	Root	% Plants Nodulated	% Plants Mycorrhizal	% Root Length Mycorrhizal
12	65	+	210[a]	119[a]	80	100	25 ± 17
		-	281[b]	143[a]	100	0	0
	150	+	667[d]	374[c]	100	100	19 ± 17
		-	441[c]	193[b]	40	0	0
20	65	+	706[a]	324[a]	80	ND	ND
		-	845[a]	340[a]	80	ND	ND
	150	+	2396[c]	1466[c]	100	100	25 - 50
		-	1312[b]	600[b]	100	0	0

[1] Data analyzed by two-way ANOVA. Data for 12 week shoot weights required a LO transformation. MSE's for 12 week shoot and root data and 20 week shoot and root data are .0059, 948.15, 4287, and 2264, respectively. Significant interactions were detected in each age group, consequently Scheffé multiple contrasts were applied to individual means. Values in each column for each age class followed by the same letter are not significantly different ($p \leq 0.05$).

Table 15. Growth of silver-berry as influenced by soil temperature and inoculation with mycorrhizal and N_2-fixing symbionts. Seedlings were grown in a growth chamber for 13 weeks.[1]

Plant Measurement	Soil Temperature (°C)	Inoculum Source			
		Uninoculated	Undisturbed Woodland	Silver-berry Soil	*Glomus aggregatum*
Shoot height (cm)	16	11.1^{bc}	12.4^{c}	9.5^{ab}	9.3^{a}
	26	15.3^{d}	23.9^{e}	20.6^{e}	12.3^{c}
Root collar diameter (mm)	16	2.2^{bc}	2.2^{bc}	1.9^{a}	1.9^{a}
	26	2.1^{ab}	2.8^{d}	2.4^{c}	1.9^{a}
Shoot weight (mg)	16	319^{b}	357^{b}	238^{a}	237^{a}
	26	485^{c}	966^{e}	727^{d}	369^{b}
Root weight (mg)	16	180^{a}	137^{a}	159^{a}	130^{a}
	26	318^{bc}	424^{c}	290^{b}	179^{a}
S/R ($\bar{x} \pm SD$)	16	2.0 ± 0.5	2.8 ± 0.6	1.7 ± 0.4	2.1 ± 0.6
	26	1.6 ± 0.4	2.5 ± 0.6	2.8 ± 0.5	2.2 ± 0.5

[1] Data analyzed by a two-way ANOVA. Shoot height, shoot weight, and root weight data required a LO transformation. MSE's for shoot height, root collar diameter, shoot weight, and root weight are .00477, .06803, .01097, and .01377, respectively. Differences detected by Scheffé multiple contrasts for pairwise comparisons. Values for each measurement followed by the same letter(s) are not significantly different ($p = 0.05$).

degree as inoculation with woodland soil. Inoculation with Glomus aggregatum pot culture soil resulted in seedlings which were significantly smaller than the uninoculated controls. Shoot/root ratios ranged from 1.6 to 2.8 with the biggest, healthiest seedlings attaining a S/R of 2.5.

Almost all the seedlings in the soil-inoculated treatments became nodulated, regardless of temperature (Table 16). Some of the seedlings in the uninoculated and Glomus-inoculated treatments at 26°C also became nodulated, presumably due to contamination from the soil treatments, but the weight of nodules produced was minimal. Nodule weight produced per plant in the soil-inoculated treatments was 5 to 11 times greater at 26°C than at 16°C. Inoculation with woodland soil resulted in more nodule production per plant than inoculation with silver-berry soil.

All of the inoculated seedlings, at both temperatures, developed mycorrhizae. However, the % mycorrhizal root length in the soil-inoculated seedlings was significantly greater at 26°C than 16°C. There was no significant effect of temperature on % mycorrhizal root length for seedlings inoculated with G. aggregatum.

In this experiment, the largest seedlings with the best mycorrhizal and nodule development occurred in the 26°C, woodland soil-inoculated treatment.

4.3.4 Use of Soil, Nodule and Pure Culture Inocula for Introducing N_2-Fixing Frankia to Containerized Silver-berry

After 18 weeks growth, the tallest (24-27 cm), heaviest (1.1 - 1.3 g shoot wt) silver-berry seedlings with the biggest (0.44 - 0.65 g) and most heavily nodulated (170 - 200 mg/plant) root systems were produced in the treatments inoculated with soil from beneath wild buffalo-berry, crushed silver-berry nodules or crushed silver-berry nodules treated with polyvinyl pyrrolidine (Table 17). Plants inoculated with Frankia ordered from Rhizotec successfully formed nodules but this was not reflected in shoot heights and shoot and root weights which were not significantly different from the uninoculated controls. No nodulation occurred in plants inoculated with Frankia which had been isolated from buffalo-berry. Treatment of

Table 16. Effect of soil temperature and inoculation on mycorrhizal and nodule development in silver-berry grown in a growth chamber for 13 weeks.[1]

Plant Measurement	Soil Temperature (°C)	Inoculum Source			
		Uninoculated	Undisturbed Woodland	Silver-berry Soil	*Glomus aggregatum*
% seedlings with nodules	16	0	90	100	0
	26	30	100	100	90
Nodules plant^{-1}	16	0	5^{a}	19^{b}	0
	26	0.5	33^{bc}	61^{c}	6
Nodule weight plant^{-1} (mg)	16	0	53^{b}	18^{a}	0
	26	10	274^{d}	200^{c}	2[illegible]
% seedlings with mycorrhizae	16	0	100	100	100
	26	0	100	100	100
% mycorrhizal root length	16	0	55^{ab}	30^{a}	76^{bc}
	26	0	97^{c}	80^{bc}	96^{c}

[1] Data was analyzed by a two-way ANOVA. Nodule number and weight data required LO and SQRT transformations, respectively. Uninoculated and *G. aggregatum* data were excluded from the nodule nuumber ANOVA and the uninoculated data were excluded from the mycorrhizal root length ANOVA. MSE's were .09578, 5.8393, and 382.9 for nodule number, weight, and mycorrhizal root length, respectively, Differences were detected by Scheffé multiple contrasts for pairwise comparisons. Values for each measurement followed by the same letter(s) are not significantly different ($p = 0.05$).

Table 17. Use of soil, nodule, and pure culture inocula for promoting growth and nodulation of container-grown silver-berry.[1]

Inoculum Type and Source	Height (cm)	Root Collar Diameter (mm)	Shoot Weight (g)	Root Weight (g)	S/R Ratio	Nodule No. Plant^{-1}	Nodule Wet Weight (mg plant^{-1})
Control	14.8^{ab}	2.0^{a}	0.44^{ab}	0.24^{ab}	2.0±0.7	0^{a}	0^{a}
Frankia pure culture (Rhizotec Labs.)	18.1^{ab}	2.5^{abc}	0.66^{ab}	0.28^{abc}	2.3±0.5	8^{abc}	124^{bcd}
Frankia pure culture (buffalo-berry)	18.4^{b}	2.4^{ab}	0.69^{b}	0.37^{bcd}	2.0±0.4	0^{a}	0^{a}
Soil (RRTAC silver-berry)	14.5^{ab}	2.2^{a}	0.53^{ab}	0.24^{ab}	2.3±0.6	3^{ab}	90^{bc}
Soil (wild buffalo-berry)	23.6^{c}	3.0^{cd}	1.10^{c}	0.44^{cd}	2.6±0.7	17^{c}	180^{d}
Nodules (Vaartnou silver-berry)	23.9^{c}	2.9^{bcd}	1.05^{c}	0.45^{cd}	2.5±0.8	12^{bc}	201^{d}
Nodules (RRTAC silver-berry)	13.9^{a}	2.0^{a}	0.41^{a}	0.18^{a}	2.4±0.5	5^{ab}	56^{ab}
Nodules: PVP-treated (RRTAC silver-berry)	26.5^{c}	3.1^{d}	1.29^{c}	0.65^{d}	2.2±0.8	16^{c}	173^{cd}

[1] Data analyzed by one-way ANOVA. Shoot and root weight data required SQRT and LO transformations respectively. MSE's for height, root collar diameter, shoot weight, root weight, nodule number and nodule weight are 5.96, .1012, 9.864, .02004, 26.54, and 2404, respectively. Differences detected by Scheff@ multiple contrasts for pairwise comparisons. Values in each column followed by the same letter(s) are not significantly different. S/R ratios are means ± SD.

nodules with polyvinyl pyrrolidine significantly improved nodule formation and growth. Nodules were also formed on plants inoculated with soil from beneath silver-berry planted in the RRTAC pad plot, but lack of stimulation in shoot and root growth suggests the nodules were ineffective at the time of sampling.

4.3.5 Effect of Inoculation Method and Time on Nodule and Mycorrhizal Development of Buffalo-berry

Although inoculation with soil or soil slurry did not significantly affect shoot height or branching, shoot and root weights were significantly greater in the soil mixture treatment than in the soil slurry or uninoculated treatments (Table 18). Plants in both the soil mixture and soil slurry treatments developed nodules but nodule weights/plant were greatest on seedlings in the soil mixture. Only plants inoculated by mixing woodland soil into the planting medium became mycorrhizal.

The age (2, 3, 4, 5 weeks) of the seedlings when soil slurry inoculum was injected into the containers did not have a significant impact on shoot height, branching, shoot weight, root weight and nodule development (Table 19). None of the seedlings inoculated with soil slurry became mycorrhizal, regardless of seedling age.

Based on these data, it appears that mixing soil with a high symbiont inoculum into the planting medium prior to planting is the most effective means for inoculating containerized seedlings.

4.4 FIELD TRIAL TO TEST GROWTH RESPONSE OF INOCULATED SILVER-BERRY AND BUFFALO-BERRY

4.4.1 Pre-Planting Symbiont Status of Inoculated and Uninoculated Silver-berry and Buffalo-berry

The mycorrhizal and nodulation status of the silver-berry and buffalo-berry grown in the University of Calgary greenhouse and outplanted on the University of Calgary reclamation plots adjacent to the RRTAC soil reconstruction-woody plant experimental area on the Syncrude lease are presented in Table 20. Both silver-berry and buffalo-berry were small and underweight compared with the seedlings surveyed from

Table 18. Use of soil and soil slurry for promoting nodulation and mycorrhizal development in container-grown buffalo-berry.[1]

Measurement	Inoculum Source		
	None	Woodland Soil Mixture	Woodland Soil Slurry
Shoot height (cm)	17.9[a]	22.9[a]	20.7[a]
Branches seedling^{-1}	6.1[a]	9.3[a]	5.4[a]
Shoot weight (g)	1.14[a]	2.06[b]	1.21[a]
Root weight (g)	0.43[a]	0.65[b]	0.34[a]
S/R ratio	2.7±0.4	3.4±1.00	3.9±0.8
Nodule weight (g wet plant^{-1})	0	0.25±0.09	0.095±0.07
Mycorrhizal root length (%)	0	25±20	0

[1] Data analyzed by one-way ANOVA and differences detected by Scheffé multiple contrasts for pairwise comparisons. Shoot height data required a LO transformation. MSE's are .0064, 14.99, .1248, and .0273 for shoot height, branches, shoot weight, and root weight data, respectively. Values in each row followed by the same letter(s) are not significantly different (p =0.05). S/R, nodule weight, and mycorrhizal values are means ± SD.

many of the commercial greenhouses (Table 8). Although 100% and 90% of the inoculated shrubs became mycorrhizal and nodulated, respectively, this was not evident in the shoot and root weights which were almost identical in the inoculated and uninoculated treatments. None of the uninoculated plants developed mycorrhizae, but nodules did develop on 20% of the silver-berry in the uninoculated treatments, presumably a result of contamination from the inoculated treatments.

4.4.2 Field Performance of Silver-berry After One and Two Growing Seasons

Percent survival of the uninoculated silver-berry after the first winter in the field was substantially better than was the case for the inoculated shrubs (Table 21). More seedlings survived in Plot 1 than in Plot 2.

Table 19. Nodule and mycorrhizal development in container-grown buffaloberry inoculated with soil slurry at various ages.[1]

Measurement	Seedling Age When Inoculated (Weeks) 2	3	4	5
Shoot height (cm)	20.7[a]	21.7[a]	20.2[a]	21.6[a]
Branches seedling^{-1}	5.4[a]	4.7[a]	4.2[a]	6.3[a]
Shoot weight (g)	1.21[a]	1.04[a]	1.14[a]	1.19[a]
Root weight (g)	0.34[a]	0.32[a]	0.36[a]	0.37[a]
S/R ratio	3.9±0.8	3.3±0.4	3.6±1.4	3.4±0.8
Nodule weight (g wet plant^{-1})	.095[a]	.076[a]	.087[a]	.072[a]
Mycorrhizal root length (%)	0	0	0	0

[1] Data analyzed by one-way ANOVA and differences detected by Scheffé multiple contrasts for pairwise comparisons. Values in each row followed by the same letter(s) are not significantly different ($p = 0.05$). S/R ratios are means ± SD.

With the exception of mycorrhizal root length, which was significantly greater in plants from Plot 2 than plants from Plot 1, there were no significant differences in plant performance and symbiont development between the two plot treatments.

After one growing season, shoots of the inoculated shrubs were 1.4 - 2.3 times taller than those of the uninoculated shrubs, while shoot weights were 3 - 7 times greater in the inoculated treatment than in the uninoculated treatment. The difference in shoot production by inoculated and uninoculated shrubs was extended into the second growing season when inoculated silver-berry were 1.6 times taller and 3.4 times heavier than their uninoculated counterparts (Tables 21, 22; Figures 3, 4). Although the root weights are probably a gross underestimate because of the difficulty in excavating entire root systems, they, nevertheless, were significantly greater in the inoculated treatment after both the

Table 20. Pre-planting mycorrhizal and nodule status of silver-berry and buffalo-berry outplanted in the University of Calgary soil reconstruction plots (with and without surficial clay). Data are means (n=10) ± SD.

Plant Species	Inoculated (+/-)	Plant weight (g dry plant^{-1})			% Plants Mycorrhizal	% Plants Nodulated
		Shoot	Root	Total		
Buffalo-berry	+	0.17 ± 0.09	0.06 ± 0.02	0.23 ± 0.11	100[1]	90
Buffalo-berry	-	0.17 ± 0.05	0.05 ± 0.02	0.22 ± 0.07	0	0
Silver-berry	+	0.50 ± 0.24	0.16 ± 0.10	0.66 ± 0.33	100[2]	90
Silver-berry	-	0.46 ± 0.15	0.14 ± 0.08	0.60 ± 0.23	0	20

[1] VAM infection ranged from 1-65%.
[2] VAM infection ranged from 1-76%.

Table 21. Plant growth, nodulation, and vesicular-arbuscular mycorrhizal development of inoculated and uninoculated silverberry outplanted for 1 year in the University of Calgary soil reconstruction plots at the Syncrude site. Data are means (n=10) ± SD.[1]

Measurement	Inoculated (+/-)	Plot 1 (tailings + peat)	Plot 2 (tailings, peat + clay)	Row $\bar{x}$
Survival(%)	+	40	16	
	-	67	47	
Shoot height (cm)	+	23 ± 7	20 ± 8	21.5^{b}
	-	10 ± 6	14 ± 4	12.0^{a}
Column $\bar{x}$		16.5^{a}	17.0^{a}	
Shoot weight (g dry plant^{-1})	+	5.35 ± 3.96	3.26 ± 2.54	4.31^{b}
	-	0.70 ± 0.83	1.01 ± 0.67	0.86^{a}
Column $\bar{x}$		3.03^{a}	2.14^{a}	
Root weight (g dry plant^{-1})	+	2.46 ± 1.77	1.49 ± 0.96	1.98^{b}
	-	0.45 ± 0.45	0.64 ± 0.46	0.55^{a}
Column $\bar{x}$		1.46^{a}	1.07^{a}	
Nodules (no. plant^{-1})	+	77 ± 79	55 ± 44	66^{b}
	-	13 ± 6	20 ± 9	16.5^{a}
Column $\bar{x}$		45^{a}	37.5^{a}	
Nodules (g wet plant^{-1})	+	1.59 ± 1.33	0.89 ± 0.70	1.24^{b}
	-	0.11 ± 0.08	0.30 ± 0.25	0.21^{a}
Column $\bar{x}$		0.85^{a}	0.60^{a}	
Mycorrhizal root length (%) (n=5)	+	56 ± 15	58 ± 14	57^{b}
	-	2 ± 2	22 ± 24	12^{a}
Column $\bar{x}$		29^{a}	40^{b}	
Shoot N (%)	+	2.69 ± 0.21	2.63 ± 0.46	2.66^{b}
	-	2.56 ± 0.37	2.12 ± 0.54	2.34^{a}
Column $\bar{x}$		2.63^{a}	2.38^{a}	
Shoot P (%)	+	0.15 ± 0.02	0.14 ± 0.04	0.145^{a}
	-	0.13 ± 0.03	0.12 ± 0.06	0.125^{a}
Column $\bar{x}$		0.14^{a}	0.13^{a}	

[1] Data analyzed by two-way ANOVA and differences detected by Scheffé multiple contrasts for pairwise comparisons. Shoot weight, root weight, nodule number and nodule weight data were LO(x + 1) transformed. MSE's are 42.45, 0.0463, 0.0238, 0.1220, 0.0174, 242.37, 0.1701 and 0.0014 for each measurement in sequential order. For each measurement row means or column means followed by the same letter are not significantly different ($p = 0.05$).

Table 22. Plant growth, nodulation and vesicular-arbuscular mycorrhizal development in inoculated and uninoculated silver-berry outplanted for 2 years in the University of Calgary soil reconstruction plots at the Syncrude site. Data are means (n = 15) ± SD.[1]

Measurement	Treatment	
	Uninoculated	Inoculated
Shoot height (cm)	34.5 ± 16[a]	56 ± 14[b]
Shoot weight (g dry plant^{-1})	10.3 ± 10.8[a]	35.4 ± 21.7[b]
Root weight (g dry plant^{-1})	2.0 ± 1.4[a]	5.8 ± 3.5[b]
Root collar diameter (mm)	5.9 ± 24[a]	10.8 ± 33[b]
Branches (number plant $^{-1}$)	15 ± 16[a]	39 ± 21[b]
Nodule weight (g wet plant^{-1})	0.7 ± 0.6[a]	2.1 ± 1.3[b]
Mycorrhizal roots (%)	43 ± 29[a]	67 ± 14[b]

[1] Data analyzed by a two sample T test. Shoot weight, root weight and nodule weight data required LO transformations. Values in each row followed by the same letter are not significantly different (p = 0.05).

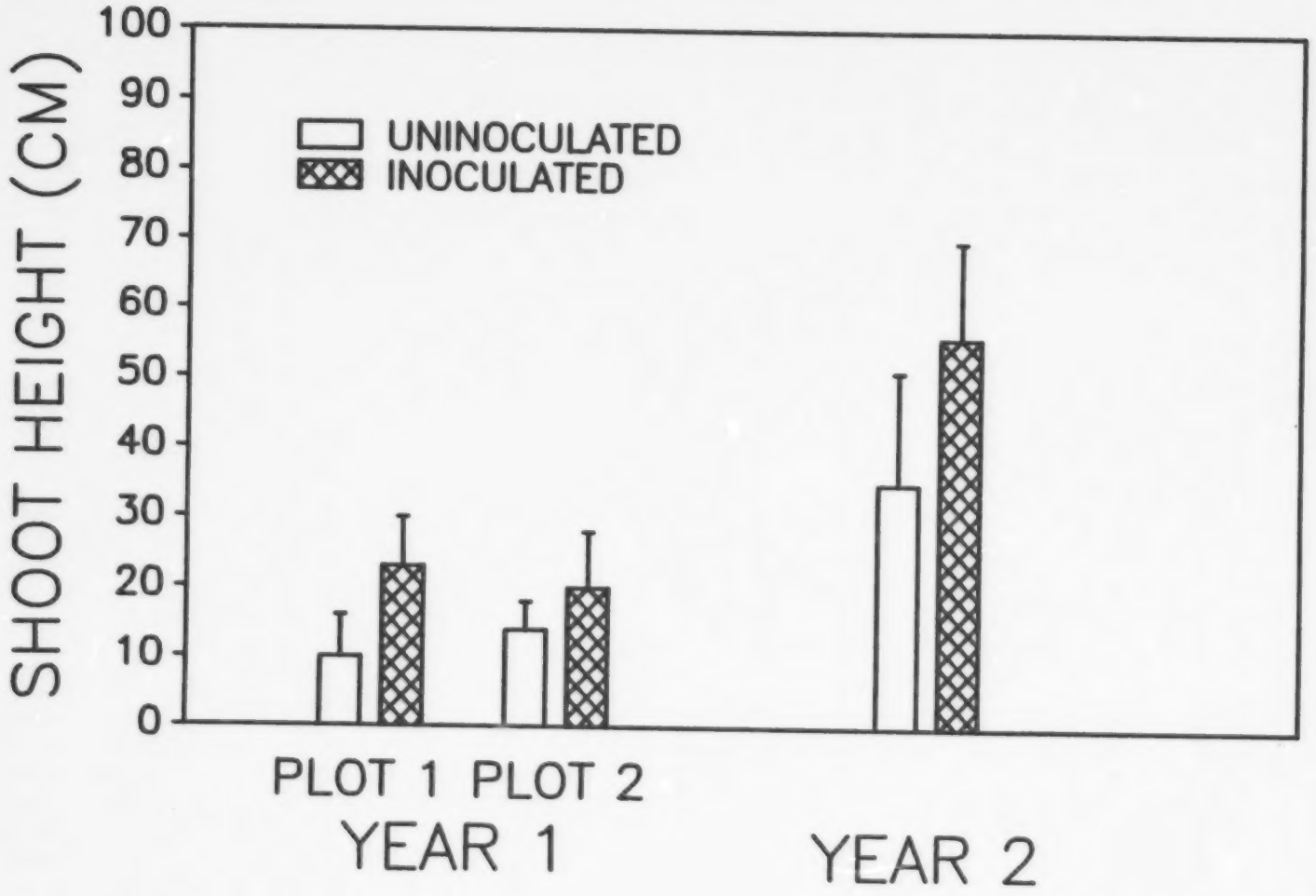

Figure 3 Shoot heights (± SD) of inoculated and uninoculated silver-berry outplanted in reconstructed soil for two growing seasons.

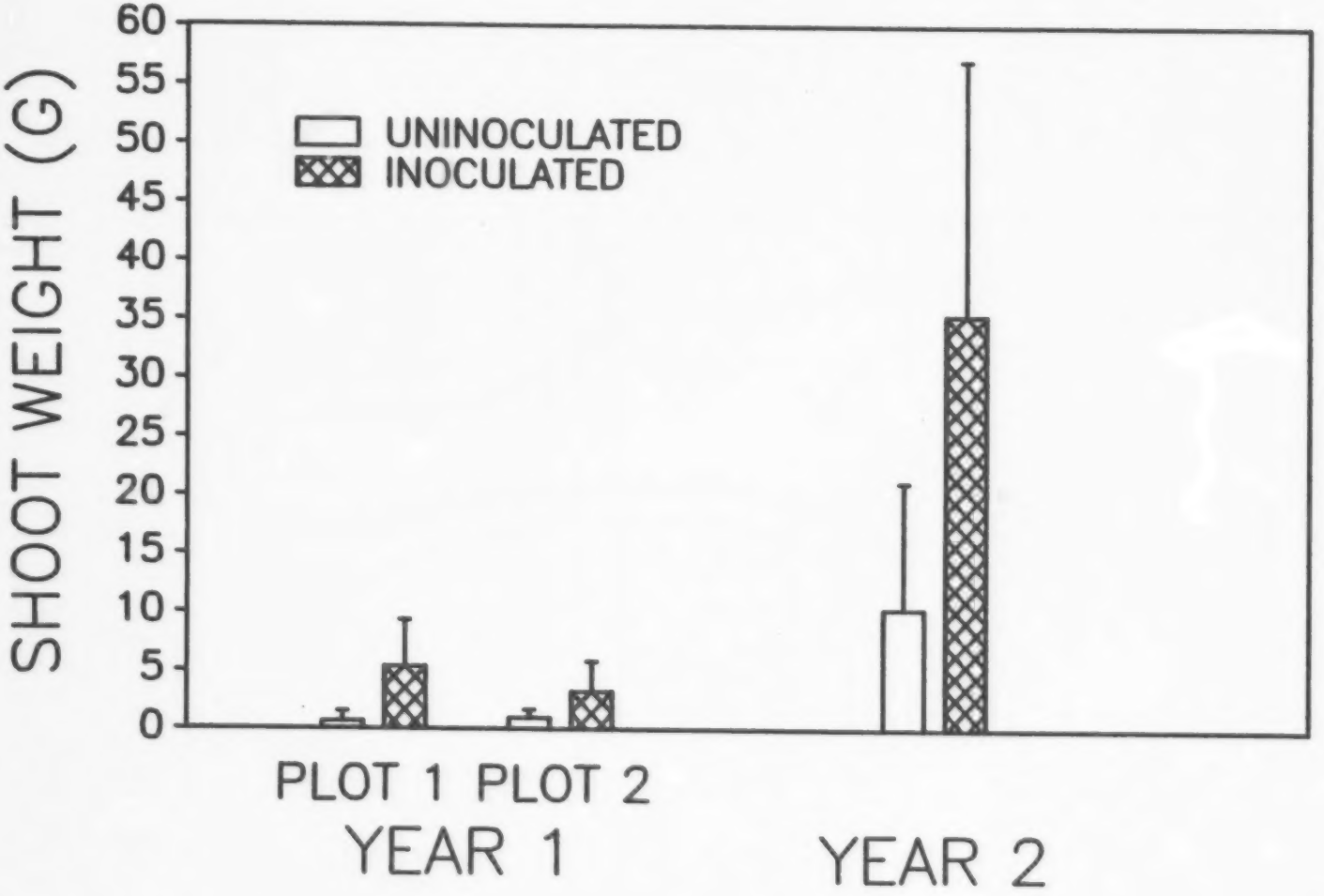

Figure 4 Shoot weights (± SD) of inoculated and uninoculated silver-berry outplanted in reconstructed soil for two growing seasons.

first and second growing seasons. Branching was also significantly better in the inoculated shrubs than in the uninoculated shrubs.

The significant growth response of the inoculated shrubs aboveground was reflected in the symbiont status on the roots belowground. The inoculated shrubs had significantly more nodules per plant and weighed an average of 3 (Plot 2) to 14 (Plot 1) times more than those which developed on the uninoculated shrubs from indigenous soil inoculum (Table 21, Figure 5). Even after two growing seasons the weight of nodules produced by the inoculated plants was 3 times greater than that produced by the uninoculated shrubs. The length of root occupied by mycorrhizal fungi was also significantly higher in the inoculated shrubs than in the uninoculated shrubs after both growing seasons (Tables 21, 22; Figure 6). After the first growing season mycorrhizal development was significantly better in the tailings sand amended with peat and clay (Plot 2) than in the sand amended only with peat (Plot 1) suggesting that the type of amendation influenced mycorrhizal inoculum potential.

After one growing season, the nitrogen concentrations in the silver-berry foliage were significantly higher for the inoculated shrubs than the uninoculated shrubs; however, no differences were detected in foliage P between the inoculated and uninoculated treatments (Table 21).

4.4.3 Field Performance of Buffalo-berry After One and Two Growing Seasons

As was the case for the silver-berry, survival of buffalo berry after the first winter was significantly less for the inoculated shrubs than the uninoculated shrubs with a greater majority of the seedlings surviving in Plot 1 than Plot 2 (Table 23).

The pattern of response of surviving buffalo-berry to inoculation was also very similar to that of silver-berry with inoculated buffalo-berry being taller (1.5 times) and having heavier shoots (3.6 - 4.5 times), roots (2.1 - 2.6 times), and nodules (1.6 - 1.8 times) and more mycorrhizal colonization (2.0 - 2.7 times) than

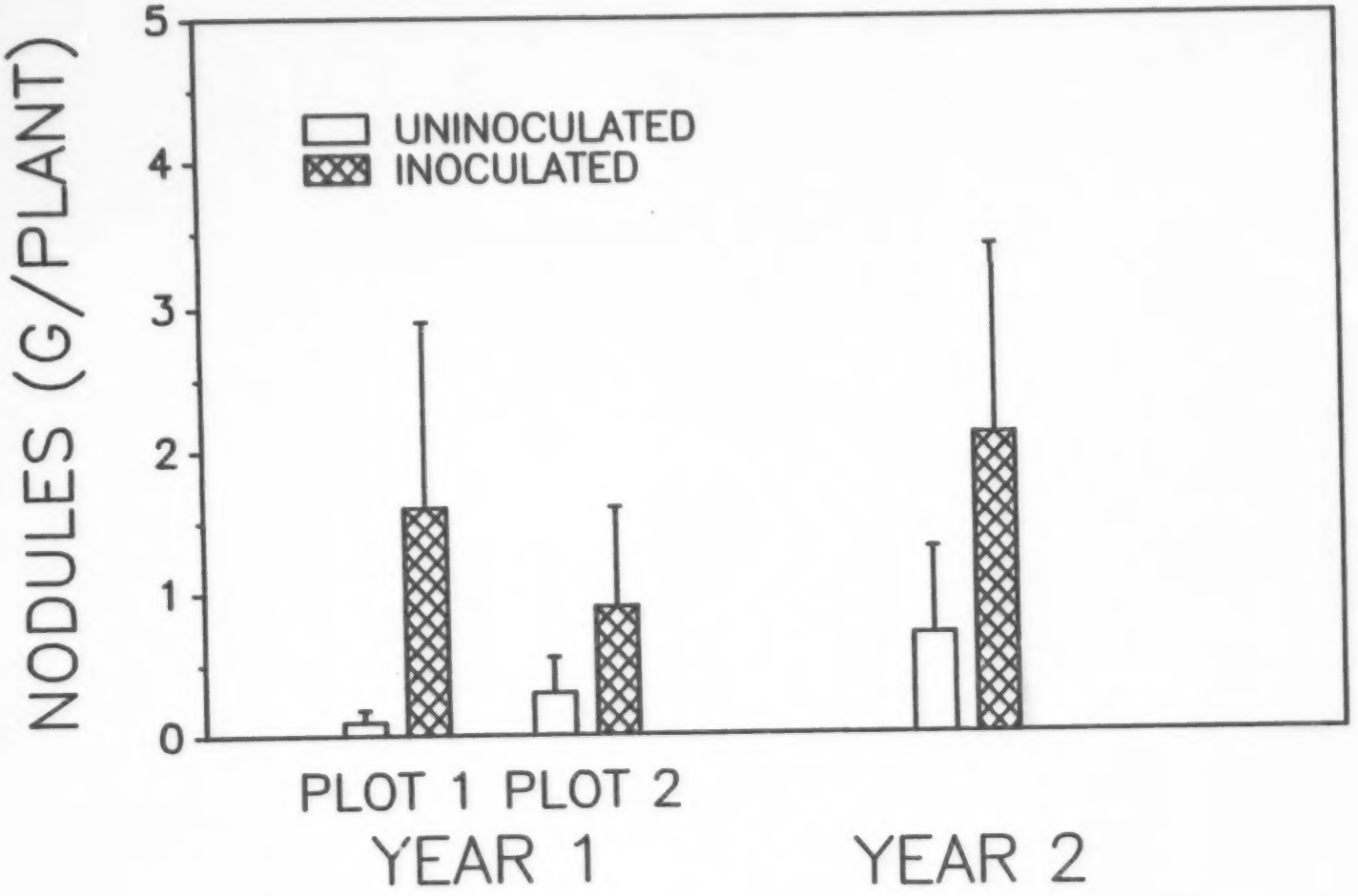

Figure 5 Nodule development in inoculated and uninoculated silver-berry outplanted in reconstructed soil for two growing seasons. Data are means ± SD.

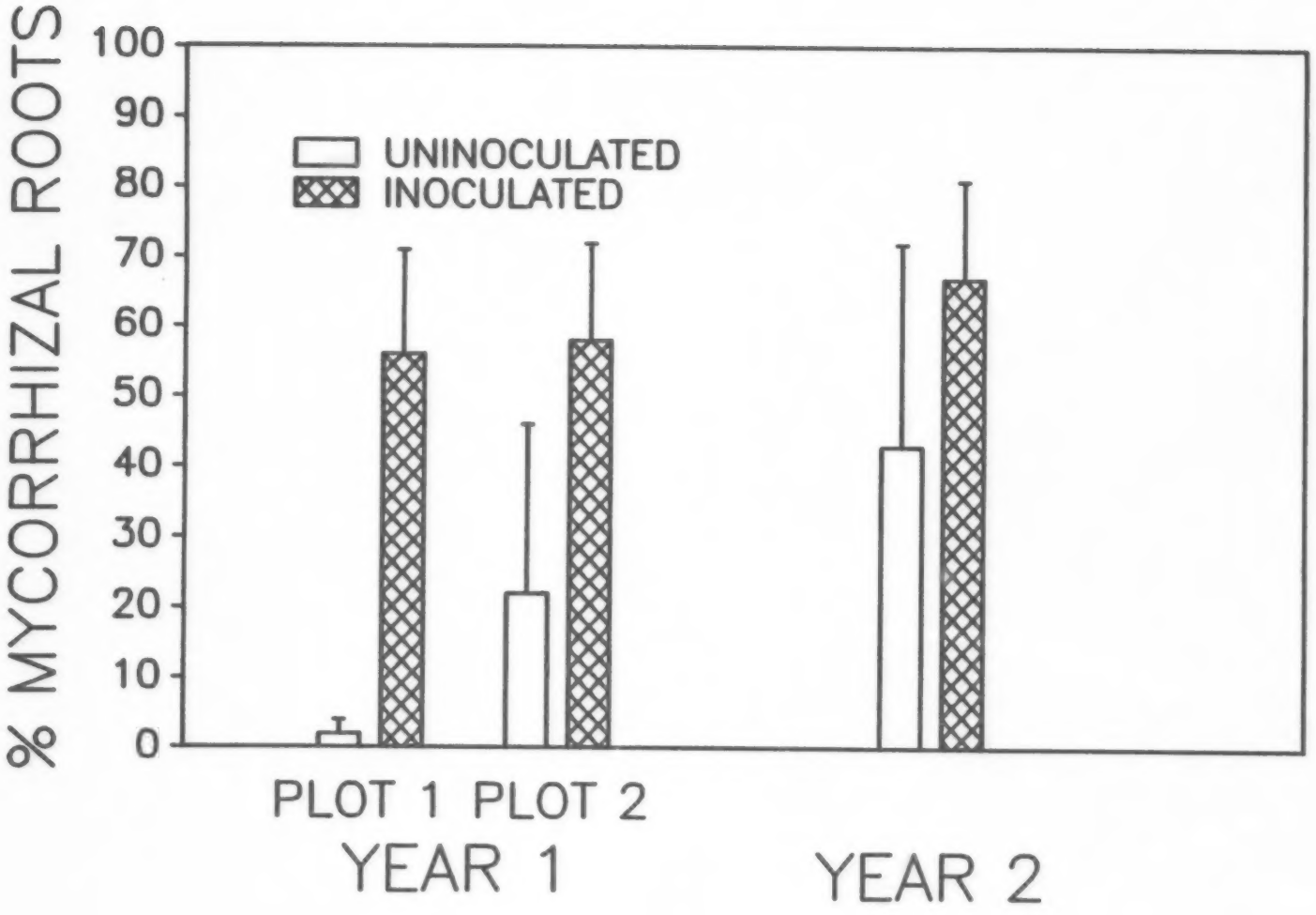

Figure 6 Mycorrhizal development in inoculated and uninoculated silver-berry outplanted in reconstructed soil for two growing seasons. Data are means ± SD.

Table 23. Plant growth, nodulation, and vesicular-arbuscular mycorrhizal development of inoculated and uninoculated buffaloberry outplanted for 1 year in the University of Calgary soil reconstruction plots at the Syncrude site. Data are means (n=10) ± SD.[1]

Measurement	Inoculated (+/-)	Plot 1 (tailings + peat)	Plot 2 (tailings, peat + clay)	Row $\bar{x}$
Survival(%)	+	48	40	
	-	75	53	
Shoot height (cm)	+	10 ± 6	14 ± 6	12.0[b]
	-	6.5 ± 2	10 ± 3	8.3[a]
Column x		8.25[a]	12.0[b]	
Shoot weight	+	0.67 ± 0.72	1.04 ± 0.91	0.86[b]
(g dry plant^{-1})	-	0.15 ± 0.07	0.29 ± 0.21	0.22[a]
Column x		0.41[a]	0.67[b]	
Root weight	+	0.30 ± 0.21	0.47 ± 0.30	0.39[b]
(g dry plant^{-1})	-	0.14 ± 0.06	0.18 ± 0.07	0.16[a]
Column x		0.22[a]	0.33[b]	
Nodules	+	22 ± 21	34 ± 32	28.0[a]
(no. plant^{-1})	-	12 ± 8	21 ± 13	16.5[a]
Column x		17[a]	27.5[a]	
Nodules	+	0.26 ± 0.26	0.41 ± 0.36	0.34[b]
(g wet plant^{-1})	-	0.03 ± 0.03	0.08 ± 0.07	0.06[a]
Column x		0.15[a]	0.25[b]	
Mycorrhizal root	+	64 ± 21	57 ± 28	61[b]
length (%) (n=5)	-	24 ± 17	28 ± 7	26[a]
Column x		44[a]	43[a]	

[1] Data analyzed by two-way ANOVA and differences detected by Scheffé multiple contrasts for pairwise comparisons. Shoot height, shoot weight, root weight, nodule number and nodule weight data were LN transformed. MSE's are 0.171, 0.657, 0.331, 0.933, 0.885 and 383.5 for each measurement in sequential order. Values for row or column means for each measurement followed by the same letter are not significantly different ($p = 0.05$).

uninoculated buffalo-berry after one growing season (Table 23, Figures 7 to 10). This pattern was carried over into the second growing season when inoculated shrubs were still significantly taller, heavier and more heavily nodulated and mycorrhizal than the uninoculated shrubs (Table 24, Figures 7 to 10).

In contrast to the silver-berry, where very few significant differences were detected between plot treatments, many of the measurements made on buffalo-berry were significantly affected by the type of amendment applied to the tailings sand. After one growing season, shoot heights and weights, root weights and nodule weights per plant were significantly greater for seedlings planted in the tailings sand amended with peat and clay (Plot 2) than for seedlings planted in tailings sand amended with peat only (Plot 1) (Table 23). Mycorrhizal infection was not affected.

4.4.4 Relationships Amongst Various Parameters Measured on Inoculated and Uninoculated Silver-berry and Buffalo-berry After One and Two Growing Seasons

Pearson product moment correlation coefficients were calculated to determine if any strong relationships existed between plant performance and the mycorrhizal/nodulation status of the roots. After one growing season there were high correlations between silver-berry shoot weights and nodule numbers (coefficient = 0.947) and shoot weights and nodule weights (coefficient = 0.912) (Table 25). Shoot productivity appeared to be more closely related to nodule status than mycorrhizal status (coefficient = 0.628). Also, percent mycorrhizal root length was not closely correlated with nodule number and weight. Neither shoot productivity nor symbiont development exhibited strong correlations with foliage nutrient (N and P) status. Correlation coefficients calculated for silver-berry data collected after the second growing season followed the same pattern as that observed for the first year data with strong correlations still present between nodule weights and shoot and root weights, but not between % mycorrhizal roots and shoot and root weights (Table 26).

A linear regression of the silver-berry shoot weights vs nodule weights for the first year data is presented in Figure 11, and

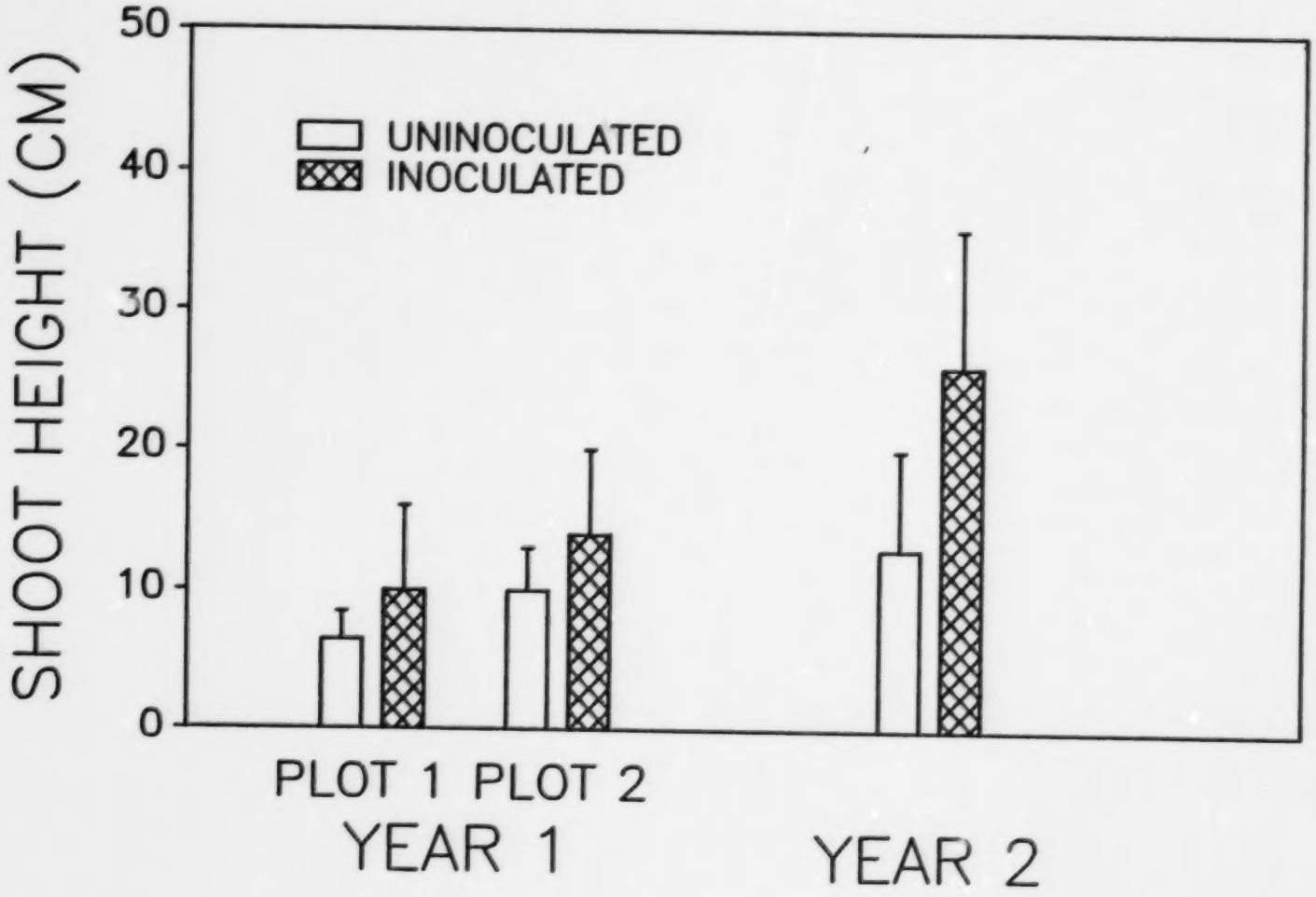

Figure 7 Shoot heights (± SD) of inoculated and uninoculated buffalo-berry outplanted in reconstructed soil for two growing seasons.

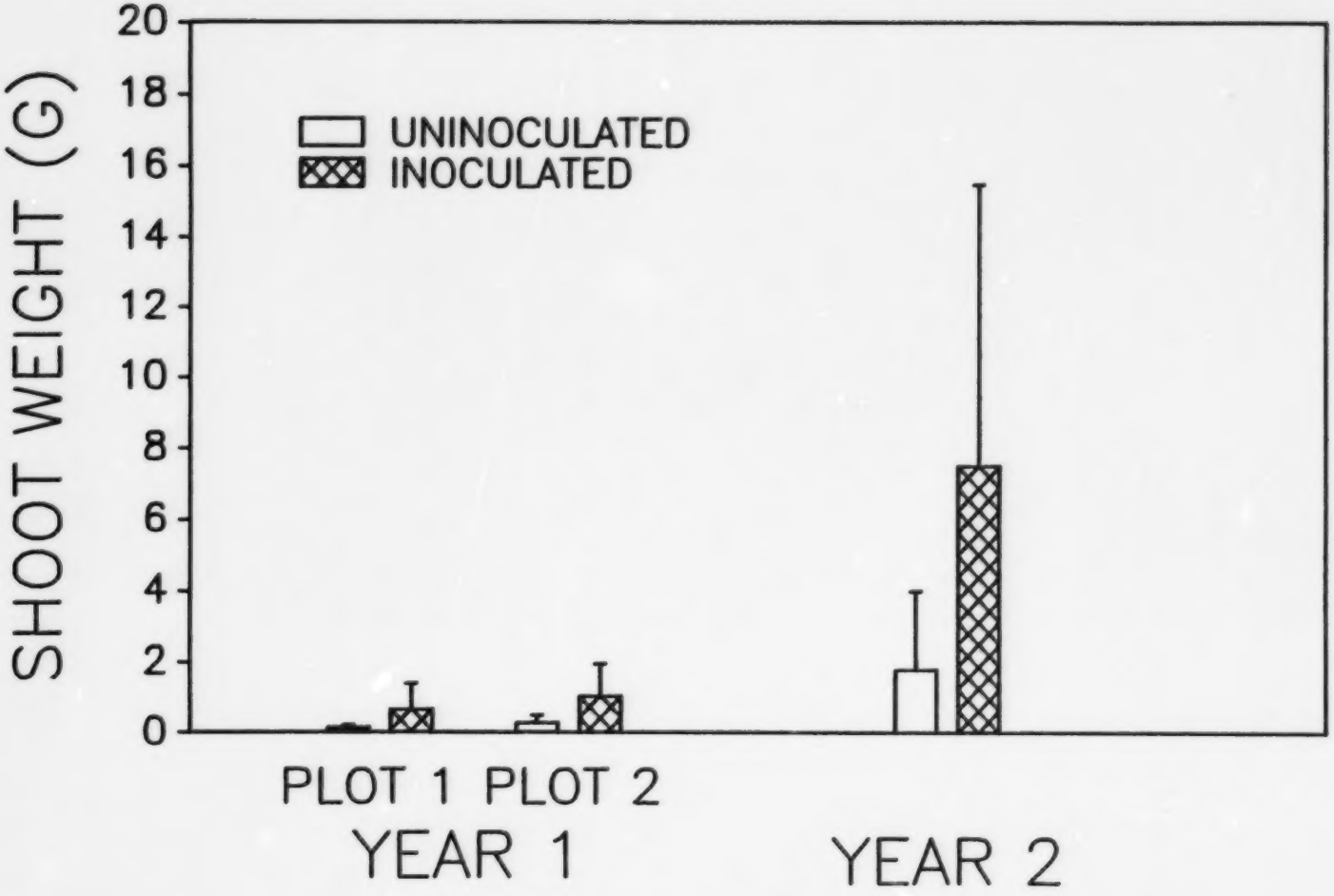

Figure 8 Shoot weights (± SD) of inoculated and uninoculated buffalo-berry outplanted in reconstructed soil for two growing seasons.

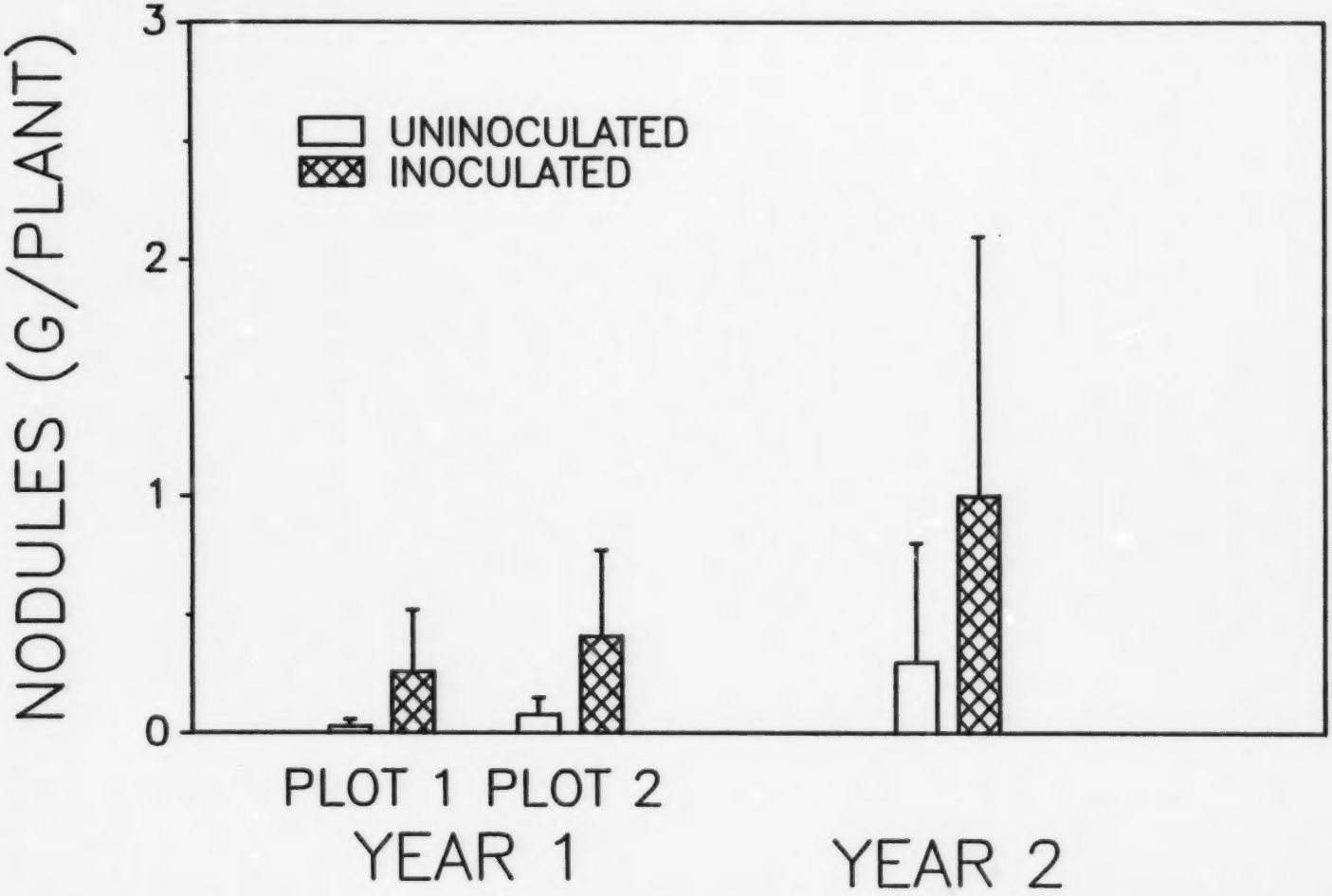

Figure 9 Nodule development in inoculated and uninoculated buffalo-berry outplanted in reconstructed soil for two growing seasons. Data are means ± SD.

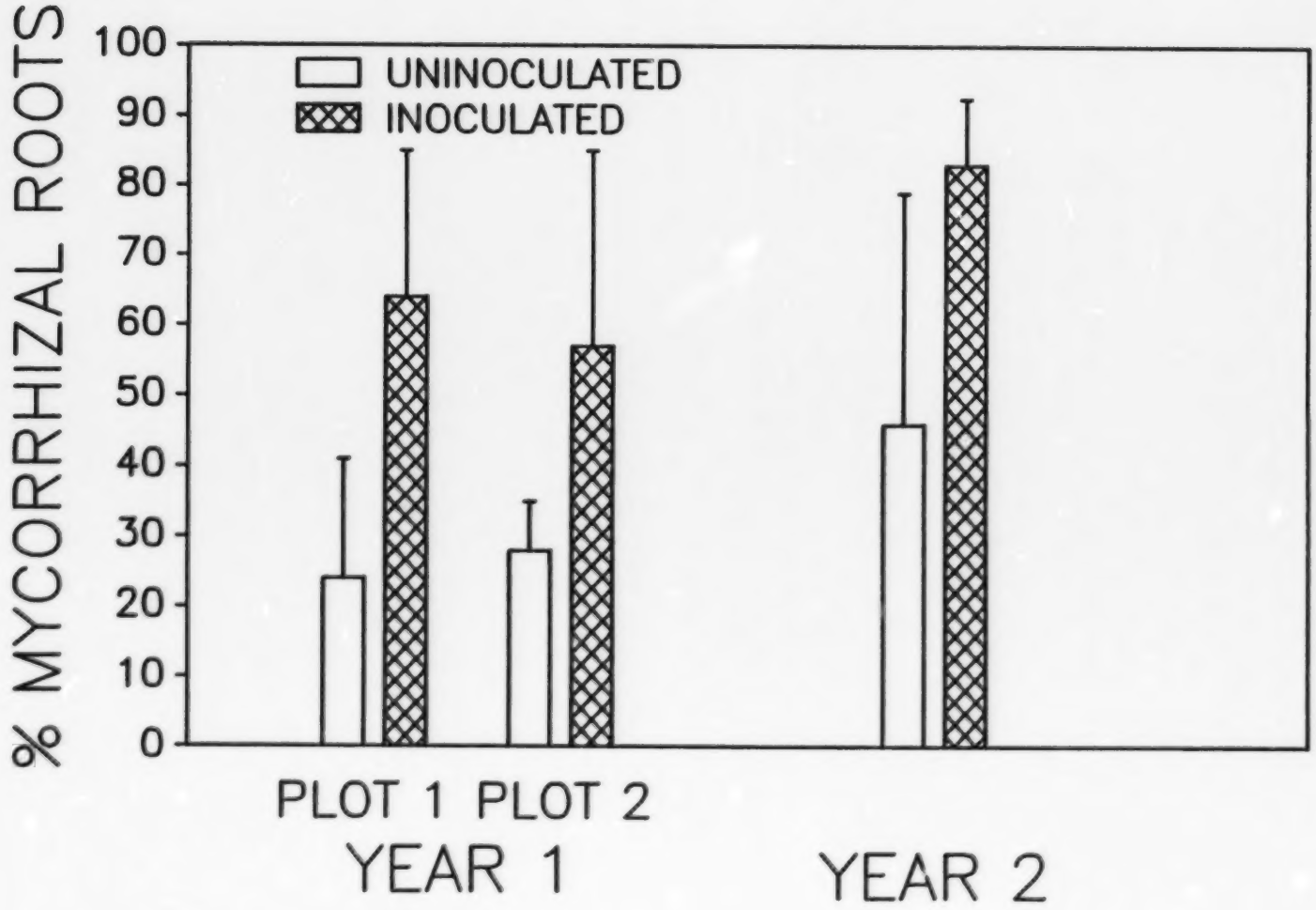

Figure 10 Mycorrhizal development in inoculated and uninoculated buffalo-berry outplanted in reconstructed soil for two growing seasons. Data are means ± SD.

Table 24. Plant growth, nodulation and vesicular-arbuscular mycorrhizal development in inoculated and uninoculated buffalo-berry outplanted for 2 years in the University of Calgary soil reconstruction plots at the Syncrude site. Data are means (n = 7) ± SD.[1]

Measurement	Treatment	
	Uninoculated	Inoculated
Shoot height (cm)	13.4 ± 6.7[a]	25.5 ± 9.9[b]
Shoot weight (g dry plant^{-1})	1.8 ± 2.2[a]	7.5 ± 8.0[b]
Root weight (g dry plant^{-1})	0.6 ± 0.5[a]	2.0 ± 1.5[b]
Root collar diameter (mm)	3.2 ± 0.9[a]	5.7 ± 2.0[b]
Branches (number plant $^{-1}$)	10 ± 9.9[a]	23 ± 26[a]
Nodule weight (g wet plant^{-1})	0.3 ± 0.5[a]	1.0 ± 1.1[a]
Mycorrhizal roots (%)	46 ± 33[a]	83 ± 9.5[b]

[1] Data analyzed by a two sample T-test. Shoot weight, root weight and nodule weight data were LO transformed. Values in each row followed by the same letter are not significantly different (p = 0.05).

Table 25. Pearson product moment correlation coefficients for various parameters measured on silver-berry grown for 1 year in the University of Calgary soil reconstruction plots at the Syncrude site.

	Shoot Weight	Shoot Height	Root Weight	Nodule Number	Nodule Weight	% Mycorrhizae	% N	% P
Shoot weight	1.0							
Shoot height	0.821	1.0						
Root weight	0.978	0.791	1.0					
Nodule number	0.947	0.711	0.949	1.0				
Nodule weight	0.912	0.793	0.916	0.950	1.0			
% Mycorrhizae	0.628	0.715	0.604	0.481	0.565	1.0		
% Shoot N	0.422	0.353	0.384	0.407	0.338	0.471	1.0	
% Shoot P	0.460	0.478	0.454	0.534	0.495	0.473	0.882	1.0

Table 26. Pearson product moment correlation coefficients for various parameters measured on silver-berry grown for 2 years in the University of Calgary soil reconstruction plots at the Syncrude site.

	Shoot Weight	Shoot Height	Root Weight	Nodule Weight	Root collar Diameter	Branches	% Mycorrhizae
Shoot weight	1.0						
Shoot height	0.810	1.0					
Root weight	0.956	0.725	1.0				
Nodule weight	0.848	0.681	0.881	1.00			
Root collar diameter	0.910	0.811	0.885	0.796	1.00		
Branches	0.946	0.815	0.862	0.832	0.821	1.0	
% Mycorrhizae	0.373	0.610	0.289	0.341	0.455	0.384	1.0

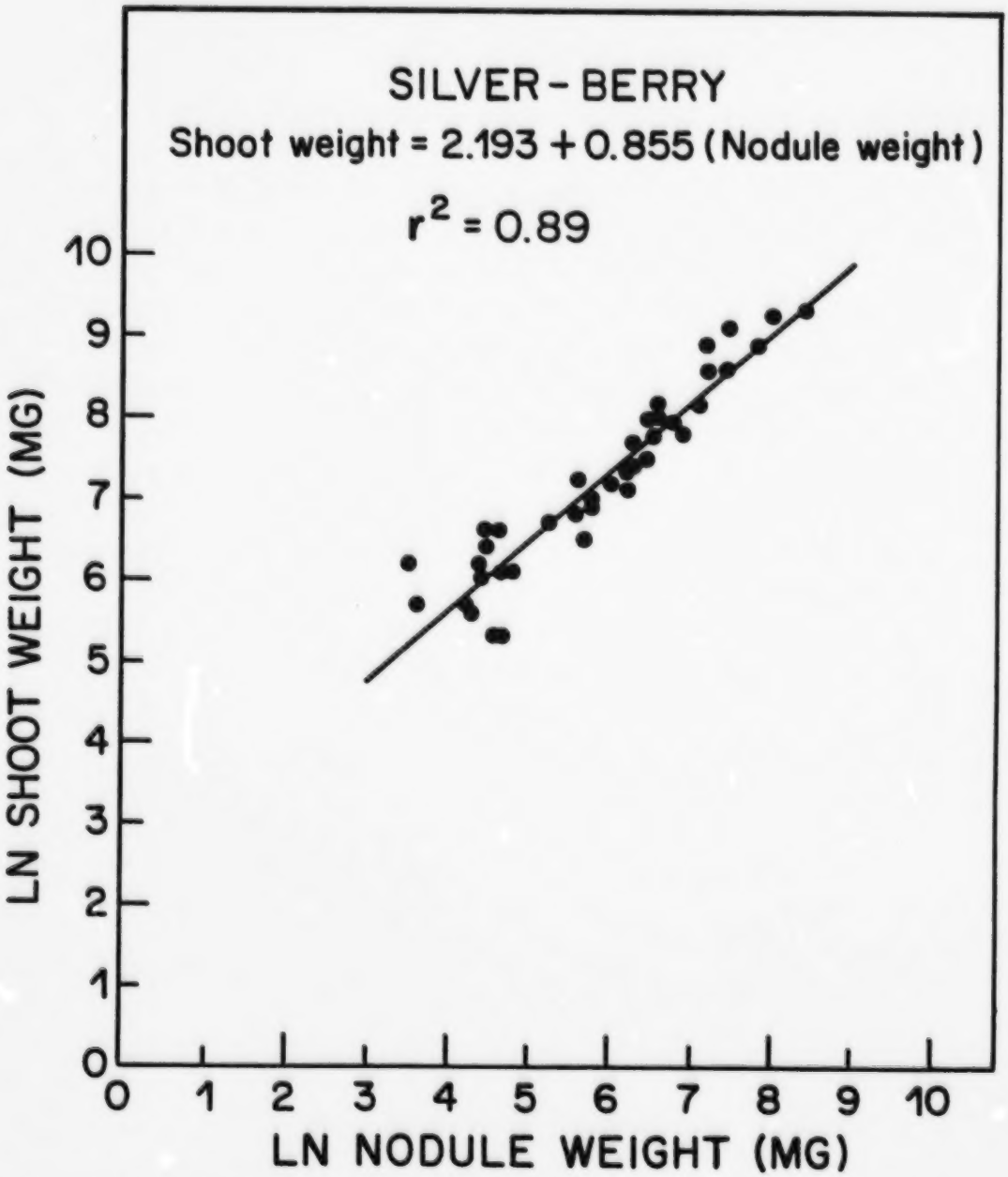

Figure 11 Linear regression of shoot weights versus nodule weights for one year-old silverberry.

illustrates the very close relationship (r^2 = 0.89) between nodulation and shoot performance. This relationship was continued into the second growing season when the regression equation was LN SHOOT WEIGHT (MG) = 1.92 + 1.11 (LN NODULE WEIGHT (MG)) and the r^2 was 0.91.

Correlation coefficients for the buffalo-berry parameters followed a very similar pattern to those calculated for the silver-berry data. Relationships between shoot weight or root weight and nodule weight were high (0.82 and 0.72 for shoot and root vs nodules, respectively) after the first growing season (Table 27) and very high during the second growing season (0.96 and 0.95 for shoot and root vs nodule weights, respectively)(Table 28). As was the case for the silver-berry, the correlation between % mycorrhizal root length and shoot weight was low (0.58 and 0.31 for years 1 and 2, respectively) as was the correlation between % mycorrhizal root length and nodule weights (0.57 and 0.26 for years 1 and 2, respectively). The close relationship between nodulation and shoot production of buffalo-berry during the first growing season is exemplified in the linear regression presented in Figure 12. The relationship during the second growing season was even stronger with the regression equation being LN SHOOT WEIGHT (MG) = 1.97 + 0.996 (LN NODULE WEIGHT (MG)) and the r^2 being 0.93.

Table 27. Pearson product moment correlation coefficients for various parameters measured on buffalo-berry grown for 1 year in the University of Calgary soil reconstruction plots at the Syncrude site.

	Shoot Weight	Shoot Height	Root Weight	Nodule Number	Nodule Weight	% Mycorrhizae
Shoot weight	1.0					
Shoot height	0.731	1.0				
Root weight	0.934	0.616	1.0			
Nodule number	0.824	0.777	0.719	1.0		
Nodule weight	0.973	0.660	0.956	0.753	1.0	
% Mycorrhizae	0.575	0.426	0.453	0.471	0.566	1.0

Table 28. Pearson product moment correlation coefficients for various parameters measured on buffalo-berry grown for 2 years in the University of Calgary soil reconstruction plots at the Syncrude site.

	Shoot Weight	Shoot Height	Root Weight	Nodule Weight	Root collar Diameter	Branches	% Mycorrhizae
Shoot weight	1.0						
Shoot height	0.873	1.0					
Root weight	0.904	0.901	1.0				
Nodule weight	0.959	0.867	0.953	1.00			
Root collar diameter	0.917	0.941	0.930	0.885	1.0		
Branches	0.896	0.704	0.669	0.822	0.746	1.0	
% Mycorrhizae	0.306	0.411	0.346	0.261	0.455	0.240	1.0

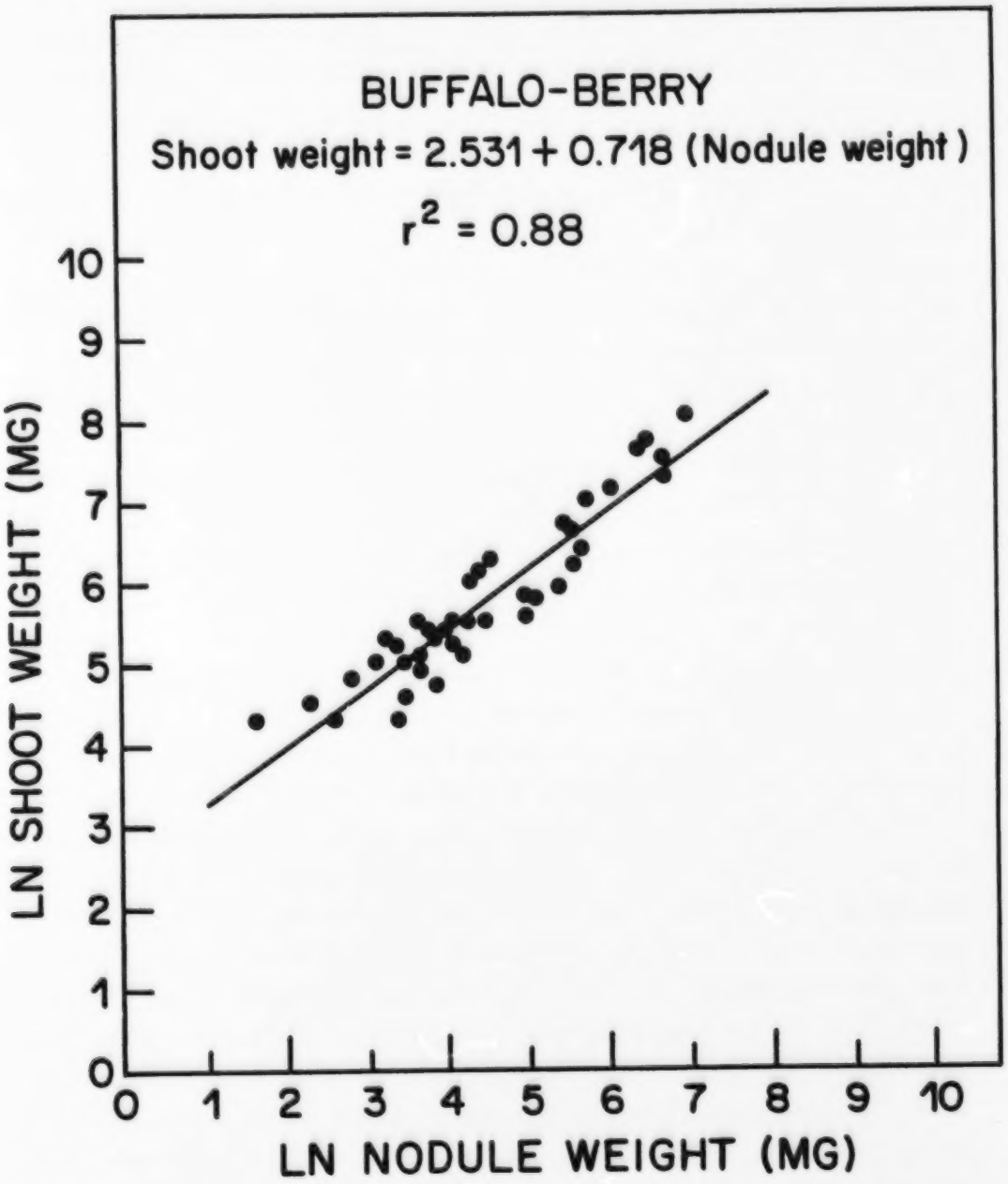

Figure 12 Linear regression of shoot weights versus nodule weights for one year-old buffalo-berry.

5. DISCUSSION

5.1 MYCORRHIZAL STATUS OF WOODY SHRUBS

Members of the Rosaceae are considered to be strictly vesicular-arbuscular mycorrhizal (Trappe, 1981); thus it was to be expected that both cinquefoil and saskatoon-berry would bear VAM-colonized roots. Silver-berry and buffalo-berry were also found to be exclusively VA mycorrhizal contrary to observations made by Rose (1980) that buffalo-berry was both VA and ectomycorrhizal. All of the plants sampled were mycorrhizal emphasizing the fact that, in the wild, the mycorrhizal condition is the norm. The high level of VAM colonization in some of the plants is indicative of a potentially high dependence by woody shrubs on the VAM symbiont.

5.2 JUSTIFICATION FOR INOCULATION OF CONTAINERIZED SILVER-BERRY AND BUFFALO-BERRY

5.2.1 The Dependency of Silver-berry and Buffalo-berry on their Mycorrhizal and N_2-fixing Symbionts

Mycorrhizal dependency is expressed as the dry weight of a mycorrhizal plant as a percentage of the dry weight of a nonmycorrhizal plant at a given level of soil fertility (Menge et al., 1982). Under the low nutrient regimes used in this experiment (plants were not fertilized and previous experiments revealed that N and P contents of muskeg peat were so low that plants responded strongly to the addition of NPK fertilizer) the mycorrhizal dependencies of silver-berry and buffalo-berry were 368% and 727%, respectively.

Soil collected from beneath buffalo-berry shrubs was used as inoculum in this study; consequently, the relative contributions of the N_2-fixing Frankia and the VA mycorrhizal fungus in stimulating plant growth could not be ascertained. However, based on the numerous studies conducted on the Rhizobium-VAM-legume symbiosis, it appears that the VAM relationship is necessary to satisfy the high P demand of the nodulation and N_2-fixation processes (Barea and Azcon-Aguilar, 1983). The high dependence of buffalo-berry and silver-berry on their symbionts suggests that early colonization by Frankia and the VAM fungi

is essential to ensure plant success in both natural and disturbed soil systems. In regards to the revegetation of oil sands tailings, it is apparent that the growth performance of buffalo-berry and silver-berry would be significantly better if the shrubs were mycorrhizal and nodulated prior to outplanting or if they were outplanted into soil with a high inoculum potential.

5.2.2 Levels of VA Mycorrhizal Inoculum in Various Soils in the Oil Sands Region and Effects of Stockpiling on VAM Infectivity

The VA mycorrhizal inoculum potential in a soil is dependent to a large extent on the mycorrhizal host plants in a specific site and on the degree of disturbance of the soil. Sites dominated by ectomycorrhizal conifers or ericaceous species (ericoid mycorrhizae) would be expected to lack VAM inoculum due to the lack of VA hosts. For example, Kovacic et al. (1984) found that VAM spores and mycorrhizal colonization were extremely low in a Ponderosa pine forest in Colorado compared with a similar forest which had been killed and colonized by VA herbs and grasses. Severe soil disturbance, such as that which occurs during mining, has been shown to significantly reduce VAM inoculum potential (Allen and Allen, 1980; Moorman and Reeves, 1979; Mott and Zuberer, 1987; Zak and Parkinson, 1982). Loss of VAM inoculum potential or reduced rates of mycorrhizal colonization as a result of topsoil storage during surface mining has also been reported (Gould and Liberta, 1981; Miller et al, 1984; Rives et al, 1980; Visser et al, 1984; Warner, 1983). Lack of VAM inoculum in a minespoil may necessitate amendation with soil having a high inoculum potential in order to ensure establishment and growth of outplanted seedlings.

Both undisturbed and stockpiled muskeg on the Syncrude lease contained negligible quantities of VAM inoculum. This is not surprising since the vegetation in undisturbed muskeg is often dominated by non-VA hosts such as tamarack, black spruce and ericaceous plants. Both tamarack and black spruce are hosts for ectomycorrhizae while the Ericales form arbutoid or ericoid-type mycorrhizae. The fungi involved in the formation of ectomycorrhizae and arbutoid or ericoid-type mycorrhizae are taxonomically very different from those which form VA mycorrhizae.

Although many of the plant species in the undisturbed muskeg were non-VA hosts, VA inoculum did occur in areas where VA hosts (grasses) were present. However, compared with mixed woodland soil where 64% mycorrhizal colonization was attained after 12 weeks growth, the infectivity potential of the peat, even when originally occupied by a VA host, was poor. Interestingly, VA inoculum, albeit low, was also present in the 50-100 cm deep undisturbed peat which was well below the rooting zone for most species. In a survey of VA inoculum in a peat deposit formed under a white spruce stand, Danielson, Zak and Parkinson (1984) also reported viable VA inoculum down to a depth of 100 cm.

No impact of stockpiling on VAM infectivity was recorded due to the lack of inoculum in the peat prior to stockpiling. Mycorrhizal infectivity was low in the stockpiled peat, but increased by 10 to 12% when the stockpile was vegetated with grass for 6 years indicating a very slow rate of increase in inoculum levels, even in the presence of VAM hosts.

The importance of the mycorrhizal symbiosis in stimulating plant growth in the peat stockpiled for 8 months was evidenced in greater shoot and root production by slender wheatgrass when a VAM fungus was artificially introduced to the peat. Some of the P deficiency symptoms (deep green to purple leaves) noted for plants grown in the uninoculated peat appeared to be partially alleviated by VAM infection. Although mycorrhizal infection stimulated plant production, 10 week old plants were still extremely small. Low rates of fertilization (15 mg N L^{-1}) substantially improved shoot production, but in the presence of fertilizer, VAM colonization did not have a significant effect on shoot weights. Numerous studies have demonstrated that, under conditions where nutrients are not limiting to non-mycorrhizal plants, growth stimulation due to mycorrhizal inoculation is lost (Abbott and Robson, 1984). This becomes particularly evident when comparing phosphate response curves (i.e. phosphorus applied versus dry weight of plant produced) for mycorrhizal and non-mycorrhizal plants (Abbott and Robson, 1984).

It appears, therefore, that undisturbed muskeg peat and stockpiled peat used in the reclamation of the Syncrude tailings dykes have neglible quantities of VAM inoculum while soil in mixed aspen white

spruce woodlands exhibit high infectivity. The lack of VAM inoculum in stockpiled peat and the potential benefits derived by the plant if inoculum is introduced into the growing medium suggests that, when revegetating tailings sand with highly symbiont-dependent shrubs such as buffalo-berry and silver-berry, attempts should be made to ensure that containerized seedlings are inoculated with their symbionts prior to outplanting. Alternatively, the tailings could be amended with inoculum-rich soil, possibly mixed woodland soil. The former approach would be more economically feasible that the second.

5.2.3 Mycorrhizal and Nodule Status of Containerized Shrubs Raised in Various Commercial Nurseries in Alberta and British Columbia

The practice of raising seedlings in containers or nursery beds offers an opportunity to manage both mycorrhizal and N_2-fixing symbionts by allowing artificial inoculation with selected symbionts prior to outplanting. Mycorrhizal inoculation of sterilized nursery soil has been demonstrated to significantly improve the growth of a range of woody plant species including fruit trees (particularly citrus), timber trees and ornamentals (Powell, 1984). As a result of this, mycorrhizal inoculation of nursery-grown citrus is now a common practice in the U.S. (Powell, 1984). The growth response of various actinorhizal plants, especially alder, to Frankia inoculation has resulted in large scale inoculation of these plants in Quebec (Périnet et al., 1985).

While in the greenhouse, containerized seedlings can become colonized by either the VA-mycorrhizal fungi or N_2-fixing bacteria residing in the planting mixture (unless it is sterilized), the atmosphere or the water. However, a survey of woody shrubs grown in Alberta and B.C. nurseries revealed that containerized seedlings seldom become mycorrhizal or nodulated during the first year of growth. If the seedlings are older than one year and have spent some time in the shadehouse or outdoors they may become mycorrhizal or nodulated but not necessarily so. The slow rates of mycorrhization and nodulation in the nurseries may be a result of a combination of factors including high fertilizer regimes (which inhibit symbiont development), a lack of symbiont inoculum and inefficient dispersal of inoculum from adjacent

inoculum sources. Regardless of the reasons for poor symbiont development in the nurseries, it can be concluded that containerized shrubs outplanted when less than one year old (which is the situation for most greenhouse operations) will be symbiont-free and, therefore, completely dependent on the inoculum present in the reconstructed soil in which they are planted. The low inoculum potential of reconstructed soil on the tailings sands dykes combined with the high dependency of woody plants on their symbionts suggests that woody species used in the revegetation of the oil sands, and possibly other disturbed areas, would benefit greatly from artificial inoculation.

Silver-berry, buffalo-berry and silver buffalo-berry surveyed in this study were often underweight and chlorotic - a condition which may have been partially due to the poor development of N_2-fixing nodules.

5.2.4 Mycorrhization and Nodulation Rates of Buffalo-berry and Silver-berry in the Greenhouse and the Field

The rapidity with which an uninoculated actinorhizal shrub seedling becomes mycorrhizal and nodulated after outplanting will determine to a large degree the benefits it will gain from the symbiosis during the first growing season. A short growing season and low symbiont inoculum potential are two factors which could reduce rates of infection to such an extent that seedlings would not benefit from their symbionts until the second growing season, if they survive the winter. Artificial inoculation would ensure that a seedling derived maximum benefit from its symbionts immediately after outplanting.

Buffalo-berry raised in amended tailings sand exhibited significantly slower rates of mycorrhization and a lower degree of mycorrhizal colonization than did seedlings grown in mixed woodland soil, presumably a result of lower inoculum levels in the tailings sand. Under ideal conditions in the greenhouse, plants in the amended tailings sand did not become mycorrhizal or obviously nodulated until eight weeks after planting. Since rates of colonization would be expected to be much slower in the field than in the greenhouse and since the growing season in the oil sands region is short, it is doubtful that containerized shrubs would gain much from the symbiosis

during the first growing season after outplanting in reconstructed soil unless they were artificially inoculated.

The relatively rapid mycorrhization of silver-berry seedlings outplanted on the Suncor dyke in June can be explained by the predominance of VAM hosts which were no doubt instrumental in raising the level of VAM inoculum in the reconstructed soil. However, compared with seedlings outplanted in a mixed woodland, seedlings on the dyke exhibited very poor nodulation over the growing season. This may have been due to a lack of Frankia inoculum in the reconstructed soil or due to very poor root growth out of the planting plug. The lack of both shoot and root growth during the term of the study is difficult to explain, and should, perhaps be investigated further. The high rate of mortality of seedlings outplanted in the Suncor plot revegetated in 1974 may have been a result of intense competition by sweet clover. Results from the field trial supported those obtained in the greenhouse and form a strong basis for considering artificial inoculation of containerized seedlings.

5.2.5 Basis for Artificial Inoculation of Containerized Buffalo-berry and Silver-berry for Outplanting on Amended Oil Sands Tailings

It appears that artificial inoculation of containerized buffalo-berry and silver-berry is justified for the following reasons:

1. Silver-berry and buffalo-berry are heavily dependent on their symbionts as evidenced by the significant growth response when they are inoculated with Frankia and VAM fungi.
2. Mycorrhizal inoculum is lacking in the reconstructed soil causing rates of nodulation and mycorrhization to be so slow that containerized seedlings would benefit tremendously if armed with their symbionts when outplanted.
3. Containerized seedlings seldom become mycorrhizal or nodulated while in the nursery, and are, therefore, virtually symbiont-free if outplanted within a year of being seeded.

5.3 DEVELOPMENT OF A GROWING REGIME FOR GREENHOUSE PRODUCTION OF MYCORRHIZAL, NODULATED SILVER-BERRY AND BUFFALO-BERRY

5.3.1 Fertilization Regimes

Many factors, including the type of growing medium, water/aeration conditions, pH, light intensity and photoperiod, temperature, container size and pesticide or herbicide applications can significantly influence the infectivity of symbiont inoculum (Menge, 1984). However, the factor which appears to have the greatest influence is the fertilization regime. It is now widely accepted that high available P levels in the soil can severely inhibit mycorrhizal infection due to an increase in the P content of the host tissue (Cooper, 1984). High concentrations of N fertilizer can also reduce mycorrhizal formation particularly if the N is in the ammonium form (Cooper, 1984; Menge, 1984). Consequently, in order to produce mycorrhizal, nodulated seedlings of suitable size and quality for outplanting, it is necessary to develop fertilization regimes which will maximize both plant production and mycorrhizal development.

Fertilizer studies on silver-berry and buffalo-berry grown in woodland soil and reconstructed soil, revealed that for both species of shrub, fertilizer applications in excess of 200 mg L 28-14-14 (i.e. 56 mg N, 12 mg P, 23 mg K) severely reduced mycorrhizal and nodule development. Plant response to fertilization was greater in the reconstructed soil than in the woodland soil, presumably because the reconstructed soil was more nutrient-poor and lacked the symbiont inoculum required to compensate for the low N and P levels. Nodulation and mycorrhization were significantly less in the reconstructed soil than in the woodland soil due to lower inoculum levels in the reconstructed soil.

High N concentrations in the soil inhibit nodulation (Bond et al., 1954; MacConnell and Bond, 1957) whereas high P concentrations in the plant inhibit mycorrhization (Cooper, 1984). Mycorrhization did not appear to be as sensitive to the concentrations of NH_4-N and P used in this study as was nodulation. At the higher fertilizer regime the dependence of the shrubs on their symbionts was lost due to greater availability of nutrients in the soil solution.

Under the conditions set forth in this study, fertilization at a rate of 200 mg L^{-1} 28-14-14 for 20 weeks produced a seedling whose shoot weight was very similar to that produced at the 400 mg rate, but whose root system exhibited well-developed mycorrhizae and nodules.

5.3.2 Container Volume

As expected, plant performance in the 150 cc containers was much superior to that in the 65 cc containers. That inoculated silver-berry grown in 150 cc containers exhibited a symbiont growth response whereas inoculated seedlings in 65 cc containers did not, is probably related to root density and the volume of soil available for exploitation by the symbionts. Baath and Hayman (1984) observed that the mycorrhizal growth response of onions was highly dependent on container size and plant density in each container. As the soil volume was reduced there was a concomitant decrease in mycorrhizal growth response. Danielson, Griffiths and Parkinson (1984) suggested that the high root density which may develop in small containers could reduce the effectiveness of the mycorrhizae, since the roots themselves would efficiently exploit the soil for nutrients with little or no dependence on the fungal mycelium. In fact, in situations such as this and where P availability is not limiting to growth, a growth depression may occur in the presence of the mycorrhizal fungi as the host and the fungus compete for plant-produced C (Buwalda and Goh, 1982). Therefore, it appears that container size and the degree to which a particular plant species can exploit the available soil volume are important factors to consider when producing mycorrhizal, nodulated seedlings for commercial purposes.

It is interesting to note that after 20 weeks growth almost all the seedlings were nodulated including those planted in the autoclaved planting medium. The nodulation of silverberry in the uninoculated (autoclaved) treatment suggests that Frankia is readily dispersed and may have been introduced by insects, particularly dipteran larvae, which were observed in the soil during the dismantling of the experiment.

5.3.3 Temperature

Temperature can have significant effects on symbiont development and alter plant growth response to the symbiosis. Maximum mycorrhizal colonization appears to occur at the point of optimum plant growth which for onion, soybean and cotton falls in a temperature range of 21 to 30°C (Furlan and Fortin, 1973; Pugh et al., 1981; Schenck and Smith, 1982; Smith and Roncadori, 1986). Below 20°C, mycorrhizal development and plant response appears to be suppressed. Similarly, infection and development by Frankia has been observed to be delayed at temperatures below 20°C with nitrogen fixation being totally inhibited at 15°C (Reddell et al., 1985). The optimum temperature for nodulation and growth of Casuarina, an actinorhizal plant found in warm temperate to tropical climates, falls in the range of 25°C to 30°C (Reddell et al., 1985).

These observations are in agreement with those obtained in this study where seedlings inoculated with woodland soil exhibited better mycorrhization and nodulation and a greater growth response at 26°C than at 16°C. In the Glomus aggregatum inoculated treatment, however, shoot weights were greater at 26°C than at 16°C but mycorrhization was not affected by temperature. This aggressive fungus caused a growth depression presumably due to an excessive drain of host photosynthate by the symbiont (Cooper, 1984).

5.3.4 Frankia Inoculum Trials

The preferred method for inoculating actinorhizal shrubs with Frankia has been the application of a liquid suspension of Frankia pure culture using either spraying or injecting techniques (Burgess et al., 1986; Fortin et al.,1983; Stowers and Smith, 1985; Vogel and Dawson, 1985). Périnet et al. (1985) compared crushed nodule and pure culture inoculum on alder seedlings and found that the use of nodule homogenates resulted in variable nodulation which was not reproducible. This was not the case in this study where inoculation of silver-berry with wild buffalo-berry soil, crushed nodules or polyvinyl pyrrolidine-treated nodules resulted in the biggest seedlings with the most heavily nodulated root systems. Seedlings inoculated with Frankia pure culture obtained from Rhizotec Labs in Quebec became heavily nodulated but this

was not manifested in improved plant growth. A slower rate of nodulation and a delay in the N_2-fixation process in this treatment may explain the lack of a growth response. It is possible a positive growth response would have occurred if the experiment had been extended. No nodule formation was evident on silver-berry inoculated with a strain of Frankia isolated from buffalo-berry. This suggests that the inoculum did not survive the inoculation treatment or that buffalo-berry Frankia may not be compatible with silver-berry. Treatment of nodules with polyvinly pyrrolidine to prevent oxidation of phenols greatly improved the effectivity of the Frankia and is strongly recommended if inoculating with crushed nodule homogenate.

The most practical source of Frankia inoculum appears to be forest floor soil removed from beneath wild buffalo-berry. This inoculum was much more effective if mixed into the planting mixture than if applied as a slurry, presumably as a result of better distribution of the inoculum. The time at which the seedlings were inoculated did not appear to be important in determining the rate and degree of nodulation suggesting that inoculum can be introduced either before or shortly after planting, whichever is most convenient. The lack of mycorrhizal development in the slurry treatments is difficult to explain, but may have been due to a reduction in mycorrhizal infectivity caused by vigorous stirring (10,000 rpm) during slurry preparation.

5.3.5 Growing Regimes for Greenhouse Production of Mycorrhizal, Nodulated Silver-berry and Buffalo-berry

Based on the foregoing results and discussion, growing regimes for the greenhouse production of mycorrhizal, nodulated silver-berry and buffalo-berry were formulated. These are presented in Tables 29 and 30. It should be kept in mind that the final heights and weights of the seedlings are dependent to a large degree on the use of highly infective and effective symbiont inoculum.

5.4 FIELD TRIAL TO TEST GROWTH RESPONSE OF INOCULATED SILVER-BERRY AND BUFFALO-BERRY

At the time of outplanting, the size of the silver-berry shrubs compared favorably with those outplanted on the RRTAC plots

Table 29. Growing regime for greenhouse production of mycorrhizal, nodulated silver-berry.

Planting Time:	March, April
Inoculum:	Silver-berry field or pot culture soil with high inoculant load; crushed nodules for N_2-fixing Frankia
Inoculum Quantity/Container:	10-15% Inoculum soil/planting mixture (V/V); inoculum mixed into planting mixture
Planting Mixture:	1/1 (V/V) Peat/Vermiculite
Container Volume:	150 cc
Grower Fertilizer:	200 mg 28-14-14 L^{-1} applied twice weekly or 56 mg N, 28 mg P_2O_5, 28 mg K_2O L^{-1} applied twice weekly
Temperature:	25-30°C
Growing Time:	12-14 weeks
Product:	24-26 cm tall, mycorrhizal, nodulated seedling with 1 - 1.2 g shoot weight

Table 30. Growing regime for greenhouse production of mycorrhizal, nodulated buffalo-berry.

Planting Time:	March, April
Inoculum:	Buffalo-berry field or pot culture soil with high symbiont inoculum levels; crushed nodules for Frankia
Inoculum/Container:	10-15% inoculum soil/planting mixture (V/V); inoculum mixed into planting mixture
Planting Mixture:	1/1 (V/V) Peat/Vermiculite
Container Volume:	150 cc
Grower Fertilizer:	200-400 mg 28-14-14 L^{-1} or 56-112 mg N, 28-56 mg P_2O_5, 28-56 mg K_2O L^{-1} applied twice weekly
Temperature:	25-30°C
Growing Time:	16-18 weeks
Product:	20-22 cm tall, mycorrhizal, nodulated seedling with 1.2 - 2 g shoot weight

(0.68 g plant^{-1}). However, the buffalo-berry seedlings were small and underweight (0.23 g plant^{-1}) compared with those outplanted on the RRTAC plots (0.77 g plant^{-1}). The poor growth exhibited by buffalo-berry, particularly during the early phases of growth, is believed to have been the result of inadequate N fertilization. It is postulated that N_2-fixing shrubs such buffalo-berry may be heavily dependent on the N_2-fixing Frankia, and therefore, require either rapid infection by this symbiont or high soil N levels to compensate for the lack of the symbiont. Supplementing the 15-15-18 fertilizer with NH_4NO_3 improved the growth of the buffalo-berry seedlings tremendously.

Both uninoculated and inoculated seedlings were of a similar size thereby justifying treatment comparisons. All inoculated seedlings developed mycorrhizae and nodules but infection was patchy possibly due to variation in the fertilizer regime.

High mortality during the first winter can be explained by insufficient hardening off and freezing weather conditions within days after outplanting. Mortality was higher for the inoculated than uninoculated seedlings, also presumably due to improper hardening off. Some studies have shown that mycorrhizal infection can reduce stomatal resistance, thereby increasing transpiration rate (Allen et al., 1981; Allen and Boosalis, 1983; Levy and Krikun, 1980) although recent investigations by Graham et al. (1987) failed to find any effect of mycorrhizal infection on the water relations of Citrus. It is possible, however, that, due to improper hardening off, the inoculated silver-berry and buffalo-berry were not in the same physiological condition as their uninoculated counterparts when outplanted. Greater stomatal conductivity and higher rates of transpiration may have increased the susceptibility of the inoculated seedlings to frost damage. It may be that mycorrhizal seedlings require a longer period of hardening off than non-mycorrhizal seedlings do. The relationship between symbiont infection and susceptibility to winter kill should be investigated in more detail.

The much superior growth performance of inoculated seedlings compared with uninoculated seedlings over two growing seasons provides unequivocal proof that pre-inoculation with mycorrhizal and N_2-fixing symbionts can, in the case of buffalo-berry and silver-berry, result in more rapid revegetion of oil sands tailings. These findings support

those of Burgess et al. (1986) where Frankia-inoculated alders significantly outperformed uninoculated alders over a three year period.

It is unknown why both inoculated and uninoculated buffaloberry were more productive in the peat and clay-amended tailings sand than in the tailings sand amended with peat only. Differences in site characteristics and soil chemical/physical properties are possible explanations.

It is difficult to determine if the symbionts introduced with the seedlings persisted and continued to colonize after outplanting; however, the much greater nodule production and mycorrhizal development in the inoculated treatments suggests that this was the case. The indigenous soil inoculum successfully infected the uninoculated seedlings, but it is postulated that symbiont inoculum potential and rates of colonization were so low that infection could not approach that in the pre-inoculated seedlings over the two year period.

The relative contributions of the mycorrhizal fungi and the Frankia to plant growth could not be discerned in this study. However, nodule number/weights exhibited a much stronger correlation with shoot weights than mycorrhizal root lengths inferring that, under the conditions of this study, shoot production was more dependent on nodule status than mycorrhizal condition. The very close relationship between shoot weights and nodule weights is emphasized in the regressions presented in Figures 7 and 8.

Vesicular-arbuscular mycorrhizae can enhance nodulation and N_2-fixation by satisfying the high P demand required for these processes (Barea and Azcon-Aguilar, 1983). Consequently, their contribution may be a subtle one and should not be underestimated. However, the relatively poor correlation between nodule status and mycorrhizal development in both silver-berry and buffalo-berry indicates that, under field conditions, other factors besides mycorrhizal status may strongly influence nodulation. Whatever the mechanisms behind the superior growth performance of the inoculated shrubs, this study has demonstrated that actinorhizal seedlings can benefit greatly over the long term from artificial introduction of their symbionts prior to outplanting. Therefore, when using actinorhizal shrubs for reclamation, and possibly for amenity and forestry purposes also, symbiont inoculation of seedlings is strongly recommended.

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Appendix Table 1. Mycorrhizal infection of slender wheatgrass grown in undisturbed muskeg and stockpiled peat (peat stockpiled for 8 months).[1]

Root Parameter	Peat Source	Sampling Depth (cm) 0-15	50-100
Total root length ($m\ L^{-1}$)	Undisturbed	545.0[a]	578.6[a]
	Stockpile	563.5[a]	623.1[a]
Mycorrhizal root length ($m\ L^{-1}$)	Undisturbed	61.5	21.9
	Stockpile	0	23.8
with arbuscules	Undisturbed	19.7 ± 35.5	5.3 ± 7.0
	Stockpile	0	3.4 ± 5.1
with vesicles	Undisturbed	4.4 ± 8.3	2.3 ± 5.1
	Stockpile	0	2.1 ± 3.0
with hyphae	Undisturbed	37.4 ± 55.3	14.3 ± 22.8
	Stockpile	0	18.3 ± 26.8
Percent infection	Undisturbed	14.5[a]	4.8[a]
	Stockpile	0[a]	3.1[a]

[1] Where possible, data were analyzed by two-way ANOVA (MSE = 322.82 and 117.05 for total root length and percent infection respectively). Values in each data set followed by the same letter do not differ significantly ($p = 0.5$). Standard deviations are included for those data which could not be analyzed.